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Investigating Stormwater Drainage Basin Sediments as a Means of Bacterial Accumulation and Transport Within a South Carolina Estaurine Watershed

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INVESTIGATING STORMWATER DRAINAGE BASIN SEDIMENTS AS A MEANS OF BACTERIAL ACCUMULATION AND TRANSPORT WITHIN A SOUTH CAROLINA ESTAURINE WATERSHED

By

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Abstract

While the presence of fecal indicator bacteria such as *Escherichia coli* in urban stormwater has been widely documented, their occurrence and persistence in sediments are not as well understood. Traditional research into bacterial contamination of surface waters has focused on overland transport of waste material and assumes fecal bacteria such as *E. coli* have short lifetimes in the environment. Recent investigations suggest that *E. coli* can accumulate in drainage basin sediments and act as a fecal bacterial reservoir within a watershed. This thesis investigated the prevalence of *E. coli* populations in the sediments and overlying waters of Withers Swash (a tidal creek) under dry and wet weather conditions. Results indicated *E. coli* persisted in the sediment environment and were resuspened into overlying waters during times of increased flow. Rain event profile results suggested the occurrence of steady state *E. coli* populations in drainage basin sediments.

Additionally, laboratory experiments investigated *E. coli* colonization of overlying waters and sediments using microcosm environments with drainage basin sediments and stormwater. These lab-based experiments also examined the importance of host sources (human and avian) to bacterial survival, expanding on the growing body of research emphasizing the importance of drainage basin sediments as they enhance the persistence and transport of the fecal indicator bacteria *E. coli* within a watershed. Each experiment used sediments of varying grain size and organic content to examine the influence of physical characteristics on bacterial prevalence. Results suggested host source of bacteria may be more important to initial bacterial colonization while physical characteristics of drainage basin sediments better explained extended *E. coli* persistence. Findings also suggested an indirect control of water column bacterial concentration by sediment type and erodibility.

Preface

This thesis is divided into four chapters. The first chapter provides a literature review and context for the presented research and outlines the objectives of these studies. The second chapter discusses methods and results of fieldwork conducted in the summer of 2012 investigating bacterial concentrations of stormwater and sediment within the Withers Swash drainage basin. The third chapter covers laboratory microcosm experiments conducted in the summer and fall of 2013, which investigated the bacterial colonization of sterile sediments and overlying waters as well as host source effects on bacterial survival. Chapter four discusses the implications of these findings and provides suggestions for further research.

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Chapter 1: Stormwater and nonpoint source bacteria pollution

1.1 Introduction

The way in which rainwater accumulates, infiltrates, and moves through a watershed represents a critical part of the hydrologic cycle. Stormwater helps maintain water levels in rivers, lakes, and streams as well as being the principal means of aquifer recharge (Winter et al., 1998). Stormwater not only provides essential freshwater for local flora and fauna but also acts as a transportation mechanism for nutrients (Wahl et. al., 1997), organic matter, and detritus (Badin et al., 2008). Stormwater streams serve as a habitat for aquatic and subaquatic organisms, providing nesting sites, shelter, food, nutrients, and a means of transport between larger habitats. Along with naturally occurring streams, stormwater provides a significant input of terrestrial waters to estuarine environments.

Expansion of human populations has lead to an alteration in stormwater function within drainage basins. In undeveloped areas stormwater is allowed to accumulate on the surface and infiltrate the sediment, becoming groundwater (Winter et al., 1998). The benefits of this process include aquifer recharge and the natural purification of water as it slowly percolates through subsurface layers (Winter et al., 1998). Development of land for human habitation often results in an increase of impervious surfaces as trees are removed and replaced with buildings and forested or grassy areas are paved. The implications of this modification in land cover include a change in quantity and quality of water flowing overland entering streams and aquifers (Morisawa and LaFlure, 1979; Arnold et al., 1982; Bannerman et al., 1993). Impervious cover has been shown to increase stormwater velocity and as a result diminish the chance of infiltration (Schneider, 1975). Due to these alterations to the topography, stormwater in urban areas often serves as a mechanism for pollutant accumulation and transport (Mallin et al., 2000, Ahn et al., 2005). Contaminants accumulated via overland flow are deposited into stormwater streams each time it rains, ultimately entering estuaries and the coastal ocean, degrading water quality (Ahn et. al. 2005). The detrimental effects to recipient waterbodies include increased erosion and sedimentation (Neller, 2006), transport of polycyclic aromatic hydrocarbons (PAHs) and heavy metals such as Pb, Cu, and Zn (Hoffman et al., 1984; Brown and Peake, 2006), nutrient loading

(Randall et al., 1978), and delivery of microbial contaminants (Geldrick et al., 1968; Qureshi and Dutka, 1979; Gannon and Busse, 1989; Chui, 1997). In light of increasingly impaired waters and the effective regulation of point sources of pollution (Arnold and Gibbons, 1996) there has been considerable research into the mechanisms and effects of stormwater as a driver of nonpoint source pollution. The research presented here focuses on bacteriological contaminants and as such this literature review will discuss only research directed at the degradation of water quality via microbes.

1.2 Literature review

Implementation of the requirements mandated by the Clean Water Act (1972) caused a shift in relative pollution contribution from primarily point sources to nonpoint sources (Karr and Dudely, 1981). Nonpoint sources of pollution are those that originate from a wide range of inputs, often over a broad geographic area (EPA, 1992). As these sources of contamination are diffuse in nature they have proven difficult to regulate. Stormwater plays a central role in the understanding of nonpoint sources of pollution and their influence on downstream waterbodies, as it is often the means of pollutant mobilization and transport (Novotny, 1995). In terms of microbiological contaminants, traditional research has focused on overland flow of runoff. Early studies by Geldrich et al. (1968) and Faust (1976) emphasized the importance of stormwater flow over the upstream watershed to downstream bacterial inputs. These studies describe bacterial deposition on the landscape via fecal matter and transport into waterbodies by stormwater runoff. Investigations into the concentration of microbial contaminants and their potential human health risks rely on the use of indicator species. Indicator species are organisms whose occurrence indicates the likely presence of other pathogens for which a direct measure is not made (U.S. EPA, 1986). The use of indicator species relies on the assumption that there is a correlation between the origin and survival of other species of interest and the indicator species. A meta-analysis by Wade et al. (2003) summarizes the findings of many years of research examining the relationship between common indicator organisms (*Enterococcus* for marine waters and *E. coli* for freshwater) with the conclusion that greater concentrations of these organisms are associated

with increased risk of gastrointestinal illnesses. Water quality assessments thus commonly use fecal indicator bacteria (FIB) to identify the recent addition of waste material from a warm-blooded organism to provide a proxy for the presence of other pathogenic species, such as *Salmonella, Cryptosporidium,* and *Campylobacter*. Testing for the range of organisms shown to pose a significant health risk to humans is expensive and cannot be done within a useful time frame. As a result FIB such as fecal coliforms, *Enterococcus*, and *E. coli* remain the primary method for inferring the presence of other harmful organisms. Using these indicators, numerous studies have concluded that stormwater, particularly in urban areas and near agricultural operations, plays a significant role in microbial degradation of water quality (Geldrich et al., 1968; Faust, 1976; Fujioka et al., 1981; Makepeace et al., 1995; Ackerman and Weisberg, 2003).

Building on early work suggesting the entire drainage basin serves as a large, diffuse source of microbial impairment, studies by Rimer et al. (1978), Arnold and Gibbons (1996), and Mallin (2000) have begun to more clearly define this relationship. Their findings not only suggest activities in the drainage area (such as urban development, agriculture, and industrial activity) influence downstream water quality but that human development and population density are directly tied to the microbial health of adjacent waterbodies. The primary driver of this relationship is thought to be the extent of impervious surface that is associated with development of an area for human use. Considerable research has explored this topic and found that the connection between increased impervious cover and degraded water quality is supported throughout the literature for a variety of aquatic environments including lakes (Byron and Goldman, 1989), streams (Goetz et al., 2003), bays (Xian et al., 2007), tidal creeks (DiDonato et al., 2009), and the coastal ocean (Ackerman and Schiff, 2003). In a comprehensive review of research exploring the relationship between impervious cover and water quality, Brabec (2002) finds a similar consensus. Her analysis of the literature emphasizes the complexity of the topic and cautions against the use of overly simple techniques for watershed management that involve single target thresholds for impervious cover. Given the ubiquity of this correlation typical watershed management approaches rely on measures to reduce impervious cover in an effort to maintain water quality. Based on her analysis of previous studies, Brabec (2002) suggests watershed

planning should also consider the actual effectiveness of mitigation efforts. She points to the importance of the location of impervious surface (not just the amount) within a watershed and the inherently different capacities of natural pervious surfaces to retain stormwater as additional factors which must be considered.

With the relationship between land use and water quality better understood, management and research efforts have since shifted to identifying host sources of microbial impairment within watersheds. Current assays for FIB used by most water quality monitoring programs are only able to determine the amount of culturable bacteria cells in a sample which will grow in a defined substrate. This information is useful for determining the relative health of a waterbody and potential public health risks for areas such as recreational beaches and fisheries. What cannot be determined from these basic tests is the host animal source from which bacteria in the waterbody originated. As a result mitigation efforts within watersheds have been difficult because sources of impairment are not easily determined. Initial attempts to define host sources relied upon microbial and phenotypic methods (Scott et al., 2002). Microbial techniques used typical indicator species (fecal coliform and fecal streptococci) and examined the ratio of their occurrence in a sample to determine human or animal origin (Geldrich et al., 1969). The presence of *Bifidobacterium* species has been used to indicate the presence of human-source waste as isolates are not typically found in animal samples (Resnick et al., 1981) and when present they are found at different frequencies for different animals (Gavin et al., 1991). Phenotypic methods have also been utilized and include antibiotic resistance (ARA) testing and a comparison of *E. coli* serotypes according to somatic (O) antigen determinants. The ARA method is based on the assumption that bacteria of human origin will be more resistant to typical human antibiotics compared to those of animal origin (Wiggins, 1996). Serogrouping of *E. coli* differentiates bacteria from different host sources based on differences in the presence of various O antigens (Parveen et al., 2001). Chemical methods have been employed which have used proxy measures for the presence of wastewater such as caffeine (Standley et al., 2000) and optical brightening agents (Hagerdorn and Weisberg, 2009), which are both found at higher concentrations in wastewater contaminated samples.

More recently genotypic techniques have been developed that aid in microbial source tracking efforts. Polymerase chain reaction (PCR) for DNA amplification has contributed greatly to the development of these methods. Using primers corresponding to repetitive DNA elements found in the genome, highly specific DNA fingerprints can be generated (Dombeck et al., 2000). These fingerprints are used to create a library of host sources against which samples with unknown bacterial isolates can be compared (Scott et al., 2002). Host-specific molecular markers are also being developed and allow for the testing and discrimination of bacterial origin using length heterogeneity PCR and terminal restriction fragment length polymorphism analysis of species of the *Bacteroides* and *Bifidobacterium* genera (Bernhard and Field, 2000). The development of real time or quantitative polymerase chain reaction (qPCR) techniques have allowed for more rapid source identification and the ability to quantify relative concentrations of markers (Staley et al., 2012) These source tracking methods are being more widely utilized as they allow for a more efficient, targeted management of sources of impairment within a watershed.

1.3 Current issues

Despite advances made in molecular detection of microbial contamination the majority of water quality monitoring is still conducted using conventional FIB. This is likely because tests for the presence and concentration of FIB are relatively inexpensive and for many purposes a concentration of FIB is all that is required. However, water resource management using FIB is only effective in protecting public health and monitoring water quality if FIB are a reliable indicator of the recent addition of waste material. The effectiveness of FIB as a proxy for pathogenic species are being called increasingly into question based on recent research into their characteristics as an indicator (Ferguson and Signoretto 2011; Korajkic et al., 2013). One necessary assumption is that FIB found in a sample could only have come from recently deposited waste material. While FIB have been historically thought to have a relatively short survival outside the gut of host organisms, research suggests some FIB may be more robust and

capable of extended persistence (Davies et al., 1995; Jamieson et al., 2003; Chandran et al., 2011; Ferguson and Signoretto, 2011). *E. coli* persistence, and in some instances replication outside the host organism, has been reported in river water (Solo-Gabriele et al., 2000) and seawater (Rozen and Belkin, 2001). Extended persistence of these bacteria relative to the species of epidemiological concern calls their effectiveness into question. Studies by Noble et al. (2001), Harwood et al. (2005), and Lemarchand and Lebaron (2011) have all reported a poor correlation between indicator bacteria and species linked to human illness such as *Salmonella* and *Cryptosporidium*. These studies suggest samples analyzed using conventional FIB may be poorly representing the level of microbial contamination.

Research has identified additional sources of FIB persistence in environments beyond the water column which may harbor and contribute FIB to water samples. Studies have shown FIB persisting in aquatic vegetation (Badgley et al., 2011) and a variety of sediment environments including streams (Jamieson et al., 2003), lakes (Chandran et al., 2011), and beaches (Boehm et al., 2009). Investigations into these environmental sources of FIB have noted significant survival and accumulation of bacteria in the underlying marine and freshwater sediments (Davies et al., 1995), tropical soils (Byappanahill et al., 2004), estuarine sediments (Jeng et al., 2005; Fries et al., 2006), and urban stream sediments (Schillinger and Gannon, 1985; Solo-Gabriele et al., 2000). FIB in the water column adsorb to suspended sediment particles, which provides advantages for survival including access to sediment-bound nutrients (Davies et al., 1995), protection from protozoan predation (Davies and Bavor, 2000), and a potential shelter from UV inactivation. Adsorbing to sediment particles of larger mass also allows bacteria such as *E. coli* to settle out of suspension during times of decreased flow. The result of bacterial adsorption and deposition could be the accumulation of substantial populations of FIB within the sediment environment. Evidence for extended FIB survival outside the host organism in sediment environments has implications for water quality monitoring. Studies by Jamieson et al. (2005) and Solo-Gabriele et al. (2000) suggest this bank of accumulated bacteria in upper sediment layers can be resuspended if agitation such as increased flow causes erosion. The result of this resuspension is a bacterial contribution to overlying waters from the sediment environment. This

too has implications for the perception of water quality based solely on FIB concentrations. Water samples may show elevated levels of FIB driven by resuspension of bacteria-laden sediments, not recently deposited waste material. As studies by Noble et al. (2001), Harwood et al. (2005), and Lemarchand and Lebaron (2011) have indicated that pathogenic species and FIB do not persist on comparable time scales, resuspension of FIB could exaggerate the potential health hazard.

1.4 Objectives and hypotheses

The goal of this research was to advance the understanding of FIB sediment interactions within a watershed. Specifically field measurements of sediment and stormwater *E. coli* concentrations were used to examine the accumulation of an FIB reservoir within sediments. These FIB populations were compared during wet and dry weather conditions in order to better understand their persistence in each matrix and ability to remobilize during elevated flows associated with storm events. Physical characteristics of drainage basin sediments were also examined for any correlation with *E. coli* concentration. For this study it was hypothesized that; 1) *E. coli* would be universally present in drainage basin sediments during wet and dry conditions, 2) drainage basin sediments would act as a source and sink for *E. coli*, 3) sediments with smaller grain size and greater organic content would harbor greater concentrations of *E. coli.* Field study description and results are described in **Chapter 2.**

Microcosm scale investigations were also conducted to more closely investigate FIB colonization of water and sediments under stagnant conditions (Water Column Colonization Experiment). Sterile overlying waters and two grain size treatments were used to examine colonization and persistence. For the Water Column Colonization Experiment it was hypothesized that; 1) *E. coli* found in sediments would colonize sterile overlying waters in the absence of agitation, 2) microcosms with smaller grain size sediments would exhibit greater *E. coli* concentrations. A Host Source Experiment was also conducted and examined the effect of FIB host source on survival and persistence in stormwater, and colonization of sterile sediments under stagnant conditions. For the Host Source Experiment it was hypothesized that; 1) *E. coli* in

stormwater would colonize sediments and persist longer in this matrix than overlying waters, 2) microcosms with smaller grain size sediments would exhibit higher *E. coli* concentrations in sediments, 3) host source would significantly affect *E. coli* persistence in each matrix. A description of microcosm experimental design and results can be found in **Chapter 3.**

Chapter 2: Evidence for the accumulation and steady state persistence of *E. coli* in subtropical drainage basin sediments

2.1 Introduction

Substantial nonpoint source pollution can be generated as stormwater accumulates within a drainage basin and moves towards a receiving waterbody (Makepeace et al. 1995). Unfortunately in many regions these receiving waters are riparian, estuarine, and coastal environments that have critical ecological and anthropogenic value (Barbier et al. 2011). Stormwater is an important component of the hydrologic cycle, aiding in recharge of lakes, streams, and aquifers (Winter et al. 1998). Stormwater not only provides essential fresh water for local flora and fauna but also acts as a transportation mechanism for nutrients (Wahl et. al. 1997), organic matter, and detritus (Badin et al. 2008). However, in urban areas as well as lands adjacent to agricultural operations stormwater may also be viewed as a means of chemical and bacteriological pollutant collection and transport. For areas experiencing considerable runoff, the ecological implications are that stormwater may act as a regular source of pollutant input. Contaminants accumulated via overland flow are deposited into stormwater streams each time it rains, ultimately entering estuaries and the coastal ocean, degrading water quality (Ahn et. al. 2005). Water quality impairment also has economic implications as many coastal cities derive significant benefit from fisheries and recreational activities associated with these waters. Coastal areas are not only the recipient of upstream drainage but are also desirable places for human habitation. As a result coastal urban environments seem to be disproportionately impacted by surface water runoff contamination (Mallin et al. 2000). These concerns for coastal areas may be exacerbated by expanding impervious cover associated with development and greater sources of pollutant input that have been shown to accompany an increasingly dense population (DiDonato et al. 2009).

Traditional investigations into bacterial contamination of surface waters have focused primarily on inputs from overland flow of waste materials (Athayde et al. 1983; Geldreich et al.

1968; Lord et al. 1987). Agricultural runoff (US EPA, 1998), pet waste (Ram et al. 2007), failing septic systems (Weiskel et al. 1996), and waterfowl (Lu et al. 2008) are among the growing list of identified sources contributing to the bacterial contamination of recipient waterbodies. Fecal indicator bacteria (FIB) are the conventional group of species that act as a proxy for the presence of more harmful pathogens in a waterbody. Epidemiological studies have shown these bacteria to be microcosm correlated with increased risk of waterborne illness (Wade et al. 2003; Zmirou et al. 2003). As a result FIB are used as a quality measure for the monitoring of recreational waters, fisheries, drinking water, and treated wastewater discharge. While overland transport of these organisms is certainly a key microbial component in the degradation of water quality, it is possible there are other sources to consider.

Currently there is a growing body of work suggesting that sediments and other matrices may play an important role in FIB prevalence and transport in a stormwater drainage system (Fries et al. 2006; Jamieson et al. 2005; Solo-Gabriele et al. 2000; Jeng et al. 2005; Ackerman et al. 2003). These studies note bacterial persistence in a variety of substrates and vegetation types (Badgley et al. 2011), associated with streams (Jamieson et al. 2003), best management practice (BMP) detention ponds, lakes (Chandran et al. 2011), and beaches (Boehm et al. 2009). The underlying sediment environment has been particularly implicated as a potential reservoir for FIB. FIB including *E. coli* have been shown to adsorb to alluvial sediment particles (Jamieson et al. 2005; Friedlander et al. 2013) where they are able to survive more readily, accumulating in the banks of a stormwater collection basin. Because FIB such as *E. coli* are not well-suited to life in the water column of a stream or estuary, adsorbing to sediment particles may increase their chance for survival (Evison,1988; Winfield and Groismon, 2003). The suggested advantages of adsorbing to sediment particles include access to sediment-bound nutrients (Davies et al. 1995), protection from protozoan predation (Davies and Bavor, 2000), and a potential shelter from UV inactivation (Fujioka et al. 1981). Adsorbing to sediment particles of larger mass also allows bacteria such as *E. coli* to settle out of suspension during times of decreased flow. The result of bacterial adsorption and settling could be the accumulation of substantial populations of FIB within the sediment environment. These sediment-bound bacteria could then be resuspended

during increased flow conditions, acting as an additional bacterial input during storms (Jamieson et al. 2005; Solo-Gabriele et al. 2000). The result of this sediment driven response could influence the perception of water quality, which may appear to have been impacted by recent fecal inputs and associated pathogens but actually experiences elevated bacteria levels due to sediment resuspension. Thus a better understanding of this bacterial reservoir and flushing phenomenon could have important implications for the way water quality is monitored. Using FIB as a proxy for other pathogenic species is only reliable if these bacteria are associated with the same sources. If indicator bacteria such as *E. coli,* the species identified as being most closely associated with waste material, have an extended persistence in sediment environments and a regular interchange with the overlying water column their efficacy as a proxy for fecal contamination may be problematic.

Here we seek to further the understanding of drainage basin sediment as a possible source/sink for *E. coli.* Specifically we aim to examine *E. coli* populations in sediment and water matrices across wet and dry conditions to determine if sediments in stormwater drainage basins act as a long-term bank and transport mechanism for *E. coli.* We employ a novel, more intensive sampling technique for rain events in an effort to profile *E. coli* populations within each matrix over the life of a storm event. We examine BMP bacterial removal efficiency as a means of investigating FIB transport via sediment particles. Additionally, we explore if physical characteristics of sediments such as grain size and organic content correlate with bacterial prevalence.

2.2 Methods

2.2.1 Site description

The study area was located in the upstream watershed of Withers Swash, a tidal estuary located in Myrtle Beach, South Carolina (**Figure 1**). Myrtle Beach typifies the expanding urban coastal environment, which often experience problems with contaminated stormwater runoff due

to increased impervious surface and development (Mallin et al. 2000). Withers Swash, with a surface area of 10.6 km^2, accounts for the largest of 29 drainage basins within the Myrtle Beach area (Guimaraes 1995). Withers Swash receives stormwater runoff from a variety of land uses including commercial facilities, residential developments, amusement parks, golf courses, and campgrounds, comprised of approximately 33% impervious cover within the watershed (Tolleson et al. 1998). Withers Swash appears twice on the South Carolina Department of Health and Environmental Control's (SCDHEC) 303(d) list of impaired waterbodies based two different intended uses. For shellfish consumption it is listed as impaired due to elevated levels of the FIB fecal coliform. For recreational use near the swash outfall it is listed as impaired using the marine water FIB standard, *Enterococci*. As there is a regular tidal exchange bacteria found in the estuary are frequently transported to the coastal environment, particularly during instances of heavy rainfall. The results of this connection with the ocean are health advisories for swimmers and permanent postings warning against swimming adjacent to the swash outfall, particularly after rainfall.

Fourteen study sites were identified in this investigation. Eleven of these were delineated to represent discrete subwatersheds within the Withers Swash drainage basin. All sites are located in upstream, open drainage ditches, excluding site 4 which is a stormwater pipe, and site 12 which is located in the tidally influenced body of Withers Swash. Four of these sites (3, 6, 7, and 9) are open streams located immediately downstream of a BMP stormwater pond outfall. Previous studies in this area classified the soils as Lakeland-Leon-Newhan, which are sandy and poorly drained and Brookman-Bladen with have a loamy surface layer and poorly drained clayey subsoil (Guimaraes 1995).

2.2.2 Sample collection

Sediment and overlying stormwater samples were collected concurrently during each sampling event from all sites. In order to investigate bacterial activity in sediment and stormwater during instances of baseline and increased flow, samples were collected during two dry and three

wet weather events. Criteria for a wet weather event were determined using hydrographs generated for each site prior to the sampling period. Based on these hydrographs it was determined that at least 0.64 cm of accumulated rainfall would be required to generate significant overland flow and thus constitute a wet event. Both wet and dry sampling events also required 72 h antecedent dry conditions to be considered an independent event (US EPA 1992). Rainfall within the study area was monitored remotely using a weather station located on 4th Avenue South, Myrtle Beach South Carolina, provided by the City of Myrtle Beach Stormwater Department.

In addition to these five independent sampling events, two storms were profiled in an effort to examine bacterial populations across the course of an entire rain event. Sediment and stormwater samples were collected at each site immediately prior to an anticipated rainfall (before the rising limb of the hydrograph) and considered “pre rain” samples. Once the criteria for a wet weather event were met, sediment and stormwater were collected again from all sites, representing the “during rain” samples. “Post rain” samples were then collected from each site after conditions had returned to approximately base flow levels, typically on the order of 24-48 h. Sampling at these three intervals was used to better understand bacterial persistence and fluctuations in population for each matrix during a storm event.

Sediment samples were collected from each site, midstream, approximately equidistant from each bank to sample consistently saturated soils and avoid the potentially confounding effects of periodic wetting on bacterial prevalence. All sites, excluding 10, maintain some water even during dry conditions. Samples were collected using a 2.5 cm diameter plastic sediment core tube, sterilized with ethyl alcohol and triple field rinsed in stormwater prior to collection. At each location the collection tube was pushed into the sediment to a depth of 5 cm, any overlying water was decanted and the sediment was placed in a sterilized polyethylene cup. A depth of 5 cm was chosen in an effort to determine bacterial concentrations of upper sediments which could be eroded and thus important as a potential contributor to water column concentrations. Three samples were collected per site and composited into a single cup.

To confirm that three subsamples provided sufficient representation of the site, system variability testing was conducted (USGS 2005). Six discrete samples were collected at three sites to assess within-site variability. Formula 1 was employed for each site using the range of bacteria concentrations in the six samples to calculate the margin of error (d) associated with collecting 3 subsamples per site.

Formula 1.

$$n = \frac{(t^2)(s^2)}{d^2}$$

where:

n = the number of required samples

t = t value based on confidence interval from T table

s = the variance in prior samples, or if unknown, $s = \frac{range}{4}$

d = acceptable margin of error (± d MPN g^{-1})

At the time of sediment sample collection, grab samples of stormwater were collected in 100 ml sterile plastic bottles buffered by sodium thiosulfate to neutralize any residual chlorine present at tidally influenced sites, which may kill bacteria. All samples were maintained at or below 4℃ and returned to the lab for bacterial ana lysis.

2.2.3 Bacteria and Sediment Analyses

2.2.3.1 Bacterial Analysis

E. coli concentrations were determined for all sediment and stormwater samples using Colilert defined substrate technology (IDEXX). Stormwater samples were analyzed and

enumerated in accordance with manufacturer recommended methods and reported as Most Probable Number (MPN) per 100 ml of sample.

For sediment bacterial analysis samples were manually homogenized using a sterilized spatula. Two 10 g aliquots were removed from each sample. One aliquot was given a 24 h drying treatment in a 100℃ oven. The resulting dry mass w as used in calculating bacterial concentration. The second 10 g aliquot was added to a sterile 250 ml glass container. Two hundred ml of sterile Milli Q high purity water were added to the container. The container was manually shaken for period of 2 min to resuspend and desorb *E. coli* bacteria and sediment particles. The mixture was allowed to settle for a period of 5 min so that larger fraction particles unlikely to harbor bacteria could settle out. The supernate from this agitation process was filtered through a 30 µm nylon mesh filter to remove the sediment fraction above this threshold. The resulting filtrate was analyzed using Colilert for FIB enumeration and reported as most probable number (MPN) per gram of dry sediment. Bacteria dilution and resuspension methods were developed based on similar work by Solo-Gabriele et al. (2000). This method was adapted to accommodate varying sediment grain sizes in the study area, which required the addition of a 5 min settling period. Resuspension and enumeration techniques were verified prior to field sampling to ensure bacteria were not lost due to resuspension methods. In brief, bacteria samples were processed in the described manner, testing a variety of shaking and settling times to determine the combination which detected the highest FIB concentration. Additionally 30 µm filters were plated on Easygel ® (Micrology Laboratories, LLC) culture plates to ensure bacteria associated with the larger sediment fraction were not lost due to the filtration process. Results of extraction testing showed minimal (<3 CFU) bacterial loss as a result of the filtration process.

2.2.3.2 Sediment characterization

Grain size and percent organic content of sediment samples from each site were examined in order to investigate their correlation with FIB concentration. Grain size analysis was conducted using a Beckman Coulter LS 13 320 Laser Diffractor. All samples were tested with and without a 48 h hydrogen peroxide treatment to determine if high organic content and aggregation

produced a significant effect on the bacteria particle size relationship. A paired samples T test was used and it was determined that samples analyzed for grain size using a peroxide treatment were not significantly different than those without ($p=0.689$). As this study was interested in the effects of sediment particles on bacterial prevalence, regardless of aggregation, particle sizes determined without a hydrogen peroxide treatment were used for all statistical analyses. Results of grain size analysis are presented using the phi scale (**Table 3**) and include standard deviation, skewness and kurtosis. The Phi scale uses a base two logarithmic scale where a large positive number indicates a small particle diameter and a small positive numbers indicates a large particle diameter. Standard deviation provides insight into the degree of sorting for a sample which describes the variance in particle diameter. Skewness indicates the degree of asymmetry for the histogram generated by grain size analysis. Positively skewed samples describe sample distributions that are skewed towards the larger end of the phi scale, indicating smaller grain size. Kurtosis describes the shape of the percent volume histogram in terms of how flat or peaked it is, providing information about how widely distributed the grain size sample is.

Percent organic content of sediment samples was determined for each site using an operational loss on ignition (LOI) procedure to estimate organic content based on combustion of volatile solids. For this technique the wet mass of a subsample of sediment from each site was determined using a mass balance. These subsamples were then given a 24 h drying treatment at 100℃ to reach a constant mass once all interstitia l water was evaporated. The dry mass of each subsample was recorded and samples were then ignited in a muffle furnace at 500℃ for a period of 6 h (ASTM D2974-07a). Mass after ignition was determined and this process of igniting and weighing was repeated until all organic content had been oxidized and samples again reached a stable mass. Organic content for sediments from each site were determined as a percentage of the total dry mass for all statistical analyses.

2.2.4 Sediment and stormwater normalization

To compare bacteria populations between sediment and stormwater matrices a normalization calculation was employed to bring all bacteria measures to a common unit. A modified version of the calculation proposed by Badgley et al. (2011) was used to estimate FIB populations in terms of surface area (CFU m^{-2}) based on stormwater and sediment concentrations and characteristics. The following formulas were used to determine bacterial population density for sediment (**Formula 2**) and stormwater (**Formula 3**) samples.

Formula 2.

$$SED_{m^2} = (10^4) * (SED_g) * (SED_{de}) * (SED_{dn})$$

where:

SED_{m^2} = *E. coli* concentration (CFU m^{-2})

SED_g = *E. coli* concentration (CFU g^{-1} dry sediment)

SED_{de} = Depth of *E. coli* colonization (5 cm used for all calculations*)

SED_{dn} = Sediment density (g cm^{-3})

Formula 3.

$$SW_{m^2} = (10^4) * (SW_{ml}) * (SW_{de})$$

where:

SW_{m^2} = *E. coli* concentration (CFU m^{-2})

SW_{ml} = *E. coli* concentration (CFU $100ml^{-1}$)

SW_{de} = Stormwater depth (cm)

***** Five centimeters used for depth of *E. coli* colonization in normalization calculations as this was the depth used for collection of all sediment samples

2.2.5 BMP testing

BMP efficiency testing provided an additional means of examining bacterial accumulation and transport associated with drainage basin sediment. As stormwater ponds retain water the flow rate is decreased and sediment particles are allowed to settle out of suspension. Bacteria adsorbed to sediment particles are also deposited, resulting in a reduction in downstream FIB concentrations. Multivariate analysis of variance (MANOVA) was conducted to examine the impact that the presence (n=4 site)/absence (n=10 sites) of a BMP had on sediment and stormwater *E. coli* concentrations.

2.2.6 Statistical analyses

Sediment and stormwater were sampled during two dry and three wet events. Additionally two storms were profiled with samples taken prior to, during, and after rain. Data from the two dry events as well as “pre rain” data from the two profiled storms were used and considered dry weather data. Wet event data consisted of the three discrete wet events as well as the “during rain” data collected during the two profiled storms. “Post rain” data were only used for within-site comparisons of sediment and stormwater concentrations throughout a rain event.

All statistical analyses were conducted using SPSS Statistical Software version 20. It was typically necessary to log transform *E. coli* and grain size data in order to meet normality assumptions of parametric statistical techniques. Percent organic content measurements were logit transformed, as they are percent, not measurement data. An *a priori* significance level of 0.05 is used for all tests unless otherwise stated.

The relationship between sediment particle grain size, percent organic content, and *E. coli* concentrations of sediment samples was investigated using multiple linear regression. Sediment *E. coli* values for each site were averaged for all sampling events and regressed against the two physical components (grain size and percent organic content). Average *E. coli* values for wet and dry sampling events only were also analyzed individually for correlation with grain size and organic content. Grain size and organic content effects on *E. coli* were also

examined using MANOVA to note if changes in either component significantly influenced sediment *E. coli* concentrations.

Weather effects were examined to determine if weather condition plays a significant role in determining bacteria concentration of sediment or stormwater. MANOVA was used to investigate weather effects on bacteria in each matrix. Main effects were considered at a significance level of 0.05, while between subject effects required a Bonferroni adjustment, using a 0.025 significance level.

Sediment and stormwater *E. coli* measures, normalized to MPN m^{-2}, were examined during dry and wet weather conditions to determine the relative population size of bacteria in each matrix. Determining approximate population sizes during both wet and dry weather conditions facilitated the testing of the bacterial reservoir and flushing hypothesis. Normalized data for each matrix were log transformed and a Student's T test was used to compare sediment and stormwater *E. coli* concentrations during dry conditions and then repeated for *E. coli* levels during wet conditions.

2.3. Results

2.3.1 Bacterial Enumeration

Sediment and stormwater showed considerable site to site variability with *E. coli* concentrations ranging from 1.5 to 794.6 MPN g^{-1} in sediment and 20.0 to >48392 MPN 100 ml^{-1} in stormwater (**Table 1)**. Data from the two profiled rain events (**Table 2**) showed a similar range in sediment (1.8 to 715.3 MPN g^{-1}) and stormwater (22.0 to >48392 MPN 100 ml^{-1}) *E. coli* concentrations. These findings are comparable to those reported by previous studies (**Table 4**).

2.3.2 Physical characteristics

Multiple regression analyses showed a significant positive correlation between median grain size, organic content and sediment *E. coli* concentrations during wet weather samples

(adjusted r^2= 0.396, p=0.042). Each independent factor entered the model, however grain size made the largest contribution to explaining the overall variation in sediment *E. coli* concentrations (β= 0.709, p=0.021). These findings were supported by the results of a MANOVA test for grain size or organic content effects, which suggested that these factors contribute significantly to *E. coli* variability. Both main effects (Wilks λ = 0.575, and 0.474 respectively) and between subject effects were significant for grain size and organic content, each with a p value of 0.002.

Grain size and organic content each significantly affected *E. coli* concentrations in sediment samples as evidenced by two different statistical tests. As noted, research suggests *E. coli* are more likely adsorbed to smaller diameter sediment particles. It is hypothesized that clusters of smaller particles with increased surface area and available nutrients are more likely associated with higher *E. coli* concentrations. Therefor results of regression and MANOVA analyses suggesting a positive correlation between grain size and *E. coli* concentration were interpreted as increased *E. coli* concentration associated with aggregations of smaller particles.

2.3.3 Weather effects

MANOVA results showed that weather condition (wet vs dry) has an overall effect on *E. coli* concentration (Wilks λ = 0.519, p<0.001). Examining between subject effects suggested that weather only significantly influenced stormwater bacteria levels (p<0.001), not those found in sediment samples (p=0.656). Stormwater *E. coli* concentrations were more than 7 times higher for samples collected during wet weather than dry weather (means of 1103.9 and 7898.9 respectively) while sediment concentrations did not differ significantly by weather condition when considering the entire system. This does not imply that at all sites sediment *E. coli* concentrations did not vary according to weather, but is an assessment of the system as a whole across weather conditions (**Figure 2)**.

2.3.4 Estimate of sediment and stormwater *E. coli* population distribution

E. coli populations were about two times as high in sediment samples as in stormwater when collected during dry conditions (Students T, p=0.001). During wet conditions stormwater *E.*

coli concentrations were about five times as high as those in sediment, which was also a significant difference (p=0.003). A comparison of normalized *E. coli* concentrations for each matrix is provided in **Figure 3**.

2.3.5 BMP effect on downstream *E. coli* concentrations

Main effects results from MANOVA indicated a significant BMP effect on overall *E. coli* concentrations (Wilks $\lambda = 0.887$, $p<0.001$). Between subject effects verified that samples of both sediment (p=0.004), and stormwater (p=0.014) collected from BMP outfalls had significantly lower *E. coli* concentrations. Mean sediment bacteria concentrations for sites with no BMP were about twice as high as those downstream of a BMP (182.9 and 90.8 MPN g^{-1} respectively) while stormwater samples from sites without a BMP saw concentrations that were about four times higher than those with a BMP (1164.4 and 4158.3 MPN 100 ml^{-1} respectively).

2.4. Discussion

2.4.1 *E. coli* prevalence in sediments

This study focused on the sediment environment as a potential sink and source of FIB within a watershed. The results of field sampling showed that the FIB *E. coli* persists in the benthic environment of drainage basin streams and the estuary into which these waters drain. Upper layers of sediment showed substantial *E. coli* concentrations (up to 10^3 MPN g^{-1}) during dry and wet weather conditions (**Table 1**), which were comparable to those found by previous studies (**Table 4**). The presence of *E. coli* populations in sediments during dry conditions suggests these bacteria are able to adsorb to sediment particles, allowing them to fall out of suspension during times of decreased flow. Previous studies by Chandran et al. (2011) and Craig et al. (2004) have reported prolonged bacterial survival in the sediment environment, resulting in a reservoir of sediments with dense bacterial concentrations. Our results support this hypothesis, as *E. coli* concentrations in sediment were often as high or higher during dry weather conditions

as those sampled during rain events **(Figure 5)**. These data suggest the sediment environment enhances bacterial survival for those FIB that settle out of suspension, leading to a bacterial reservoir within the drainage basin.

Normalizing *E. coli* measures to a common unit (CFU m^{-2}) allowed for further examination of *E. coli* populations in watershed sediments and stormwater. These data showed that for samples collected during dry conditions sediment *E. coli* concentrations were significantly higher than those found in stormwater, while wet conditions were associated with higher stormwater *E. coli* concentrations (**Figure 3)**. Greater bacterial densities during dry conditions supports the buildup of a bacterial reservoir via sediment deposition. The relative bacterial populations of each matrix were also compared using these normalized measures and emphasized the importance of the sediment environment to overall *E. coli* population (**Figure 4)**. While this comparison does not consider additional populations of FIB beyond stormwater and sediment (e.g. submerged aquatic vegetation) it does suggest that in parts of the system the sediment environment may account for a significant fraction of the overall *E. coli* population between rain events.

Physical characteristics of sediments such as organic content and grain size were also examined as they have been suggested to influence bacterial survival in the sediment environment. In the Withers Swash watershed grain size and organic content had a significant effect on sediment *E. coli* concentrations (r^2=0.396, p=0.042) as multiple regression results showed that each was positively correlated with *E. coli* concentration. Within this analysis grain size makes the largest contribution to explaining *E. coli* variability (β= 0.709, p=0.021), indicating that these bacteria adsorb more frequently to larger sediment particles. These results likely suggest greater *E. coli* adsorption to aggregations of sediment, which often include organic materials and nutrients. These findings are in agreement with Chandran et al. (2011) and Craig et al. (2004) who explain enhanced bacterial survival within a more organic rich sediment environment. It is possible these bacteria survive longer once adsorbed to sediments with a higher organic content as a result of access to nutrients, which may be limited in more mineral rich soils and the water column (Craig et al. 2004).

2.4.2 BMP sampling and bacterial transport

Results from our comparisons indicated that the four sites sampled at BMP outfalls had significantly lower *E. coli* concentrations than the ten sites without a BMP (**Figure 6**). Finding significant bacteria removal downstream suggests that bacteria are being transported via sediment adsorption versus freely floating cells and that increased residence times in BMP ponds are effective at removing bacteria from the water column. This finding is consistent with estimated settling times of *E. coli* cells not adsorbed to sediment particles of 0.0052 – 0.021 cm h^{-1} (McClaine and Ford 2001). Even using the most generous settling velocity estimates, freely floating *E. coli* cells would only settle 14.1 cm over the course of a month under no flow conditions. Settling times for sediment particles and the associated bacteria are more complex and vary according to particle size, however a study by Schillinger and Gannon (1985) reported 73%-86% of particles with a diameter greater than 5µm settling in 5 hours. It could be thus reasoned that this same of bacteria laden particles occurring in the stormwater ponds would occur in the stormwater streams as flow rates slow and could account for the increases in bacteria following a storm event (**Figure 5a)**.

2.4.3 Rain event profiles

Sampling sediment and stormwater throughout the course of a rain event provided greater insight into FIB activity. Dry weather sampling offered evidence of bacterial adsorption and deposition while wet weather sampling of sediments provided some evidence of FIB resuspension. Profiling an entire rain event at all sites however allowed for comparison of populations in each matrix within the same rainfall event. This provided more direct evidence of bacterial accumulation, mobilization, and population rebound. Of 27 profiles produced 18 exhibited a trend consistent with bacterial reservoir and flushing activity (**Figure 5a**). These sites contained elevated concentrations of *E. coli* in sediments immediately prior to rainfall and a subsequent decrease of bacteria during the stormwater event. A drop in sediment *E. coli* concentrations coinciding with elevated flows during rainfall were considered on a site-by-site basis using individual profiles and suggested that sediment-associated bacterial was

resuspended into the water column. This sampling technique was beneficial as it allowed for approximation of bacterial exchange between matrices using basic water quality monitoring tests.

Post rain data were examined to investigate sediment bacterial population recovery and the time frame in which deposition may regenerate an FIB bank in streambeds. Interestingly, almost 60% (16 of 27) of profiles showed sediment *E. coli* concentrations rebound within 48 h to levels similar to those observed prior to rainfall (**Figure 5a)**. Samples were collected at the end of the falling limb of the hydrograph, suggesting upstream contributions of sediment associated FIB, which settle out as flows recede. While FIB enter the drainage system via overland flow and sediment resuspension it is unlikely that bacteria which are not adsorbed to sediment particles could deposit into the benthic environment under typical conditions, due to their low density and slow settling velocity. This emphasizes the importance of sediment bound FIB between rain events to the accumulation of an FIB reservoir. Adsorbing to sediment particles likely provides a competitive advantage for sediment-adsorbed bacteria over freely floating ones and is the primary driver of FIB accumulation within a watershed. These findings suggest that *E. coli* may persist in a relatively steady state concentration between rain events based on some inherent quality of the drainage basin. Finding a rapid return to near pre rain concentrations was surprising based on numerous environmental factors including site characteristics and weather conditions, which could influence bacterial survival in the environment. As discussed earlier the study sites represent a range of both potential FIB sources and drainage basin characteristics. Thus, it should be expected that each site would respond differently to antecedent weather conditions, the magnitude of a storm event, and the features of the site, particularly the presence of BMPs. However, despite this environmental variability all 14 sites exhibited a response which fit the reservoir and flushing hypothesis during at least one of the two completed profiles. This implies that the sediment bacterial accumulation, flushing, and rebound phenomenon may be ubiquitous under a wide range of conditions.

2.4.3 Management implications

The findings of this study add to the understanding of how populations of FIB enter and persist in a watershed. Results presented here provide evidence for the presence of a long-term reservoir of FIB in drainage basin sediments that is likely resuspended, enters the water column, and rapidly rebounds to pre rain levels during dry conditions. This suggests an additional source of FIB input to overlying waters that has been unaccounted for under conventional watershed management techniques. Many water quality monitoring programs require grabs of individual samples in an effort to characterize bacteriological health, with the understanding that during storm conditions these samples represent only a snapshot of a dynamic system at the time of collection. Our findings suggest sample results are likely also influenced by sediment inputs. Sites sampled from drainage pipes may show bacteria values lower than those observed within the same branch of the watershed that were collected from streams in which sediment can be resuspended. Streambed sediments may be eroded naturally as bioturbation decreases the stability of upper layer sediments (Jones et al. 1994). Sediment disturbance may also come from anthropogenic origin as expanding impervious cover leads to increased runoff velocity and greater erosion of benthic sediments (Paul and Meyer 2001). Erosion as a result of stormwater runoff could thus have further detrimental effects if it leads to the resuspension of sediments with dense bacterial populations. Localized areas of significant sediment input could therefore make identifying sources of impairment more difficult as overland transport may be less important than long-term bacterial accumulation within streambeds.

An additional source of FIB which was not studied but should be considered are those contributed from groundwater. Drainage systems which transport water via ditches are susceptible to groundwater infiltration in areas where ditches fall below the water table. There is evidence that FIB can survive and be transported through surficial aquifers and return to surface waters as groundwater is discharged (Boehm et al. 2004; Ferguson et al. 2012). In a coastal area such a Myrtle Beach with a relatively shallow water table it is possible that groundwater comprises an additional input of bacteria during dry weather conditions. While this research did not focus on groundwater, comprehensive studies investigating all potential sources of FIB in coastal watersheds should consider this as a potential input.

These findings are epidemiologically important as *E. coli* serves as an indicator species, not the pathogenic species of concern. As stated they are only a proxy measure for the recent addition of waste material, which transmits pathogens such as *Campylobacter* and *Salmonella* shown to cause human illness. Any FIB persistence in the environment would thus impede the ability of typical bacteria tests for FIB to detect recent microbial impairment, as they are unable to distinguish between persisting and newly deposited FIB. The survival of FIB outside the host organism has been shown to correlate poorly with the survival of species for which they are an indicator (Lemarchand and Lebaron 2003; Harwood et al. 2005; Noble and Fuhrman 2001). If FIB survive in the environment on time scales that differ from those of pathogenic species they may not serve as a reliable indicator of the presence of pathogens. An additional concern is raised by the inability of culture-based bacteria tests to detect viable but non-culturable (VBNC) bacteria (Oliver 2000). FIB in this state, which are not detected by tests such as Colilert, may return to a culturable form making accurate interpretation of the true human health threat even more difficult. These findings thus question the efficacy of conventional indicator species.

A further concern is raised by the ubiquity and observed persistence of *E. coli* in sediments across the study area. These results may also suggest the possibility of an autochthonous population of FIB. Studies using genetic fingerprinting techniques have shown naturalized *E. coli* and *Enterococci* populations present in lakes (Byappanahalli and Fujioka 2004), forest soils (Byappanahalli et al. 2005), watershed sands and sediments (Solo-Gabrielle et al. 2000; Jamieson et al. 2005), and submerged aquatic vegetation (Badgley et al. 2011; Whitman et al. 2005). Evidence suggesting independent populations of these bacteria that are genetically different from those of recent enteric origin makes monitoring bacteriological water quality more difficult. As a result typical bacteria testing may be detecting a combination of recently deposited FIB, long survived FIB from sediments, and naturalized species of FIB with no way to determine the true public health hazard. Economic losses are also conceivable as recreational beaches or fisheries may be unnecessarily closed due to inflated FIB numbers associated with sediment resuspension, not waste material in the watershed.

Findings of this study provide valuable information for watershed managers. Water resource monitoring and the development of total maximum daily loads (TMDL) for pollutants should be adapted given evidence of bacterial persistence and resuspension within a drainage basin. As assessment of sustained FIB populations in sediment environments is likely required to investigate all potential sources of microbial impairment.

2.5. Conclusions

There are many factors that contribute to the bacteriological health of a waterbody. This study showed that *E. coli* persisting in the sediment environment could act as a significant source of bacteria to a watershed. A comparison of the population of *E. coli* contained in the water column and sediment indicates that sediment populations may account for the majority of the total *E. coli* on a per surface area basis, particularly during dry weather conditions. Observed decreases in sediment *E. coli* during stormwater events suggest that resuspension is mobilizing sediment bacteria into the water column. Rapid recovery of sediment *E. coli* concentrations (~48 h) to near pre-storm conditions suggests that deposition and persistence can produce steady state populations of FIB in sediments. The regulatory implications of a sediment FIB load mean that stormwater FIB concentrations may not accurately reflect the inputs of new bacterial pollution and thus the actual risk to public health.

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Table 1 - *E. coli* enumeration results for stormwater and drainage basin sediments. Field measurements of *E. coli* in drainage basin sediments and stormwater with associated error.

Sediment *E. coli* (MPN g^{-1})

Site	Dry Weather 1 (5/20/12)	95% CI (-/+)	Dry Weather 2 (6/27/12)	95% CI (-/+)	Wet Weather 1 (5/30/12)	95% CI (-/+)	Wet Weather 2 (8/28/12)	95% CI (-/+)	Wet Weather 3 (9/18/12)	95% CI (-/+)
1	851.4	277.7 / 373.3	7.5	2.6 / 3.2	266.4	86.9 / 116.9	57.8	16.6 / 21.5	7.7	2.5 / 3.3
2	139.7	48.3 / 66.5	83.9	24.1 / 30.6	53.0	14.2 / 16.4	679.2	221.5 / 644.6	48.7	8.3 / 9.6
3	21.2	4.4 / 4.9	1.5	1.1 / 1.7	47.3	12.7 / 15.5	53.3	11.1 / 12.1	16.5	4.7 / 6.0
4[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]
5	29.3	5.5 / 6.5	11.3	2.8 / 3.3	240.7	78.5 / 94.4	53.8	16.5 / 23.6	9.4	2.5 / 3.2
6	199.8	41.8 / 56.6	8.3	2.8 / 3.9	562.7	194.5 / 289.9	413.1	142.8 / 189.7	19.8	4.9 / 5.8
7	4.1	1.8 / 2.7	53.0	13.1 / 16.5	14.4	5.0 / 6.3	7.5	2.6 / 3.5	16.4	4.7 / 6.0
8	50.6	12.6 / 16.5	236.8	81.9 / 99.2	7.3	2.5 / 3.1	30.7	8.2 / 9.4	7.2	2.5 / 3.2
9	2.9	1.6 / 2.2	49.1	12.2 / 14.3	9.5	3.3 / 4.2	18.2	4.5 / 5.1	5.2	2.2 / 3.3
10[b]	[b]	[b]	[b]	[b]	146.4	50.6 / 69.8	298.9	97.5 / 130.8	28.4	7.6 / 10.1
11	6.8	2.1 / 2.7	8.5	2.5 / 2.9	794.6	259.2 / 754.2	78.4	28.6 / 41.6	100.5	36.7 / 51.0
12	743.1	285.9 /491.6	2093.1	727.7 / 1879.2	638.7	208.3 / 606.2	56.2	11.7 / 13.3	29.6	5.6 / 6.4
13	20.6	3.9 / 4.6	321.0	104.7 / 140.8	152.4	34.8 / 41.3	100.4	30.8 / 40.6	91.5	28.0 / 39.9
14	38.5	8.0 / 8.8	27.9	6.9 / 8.5	206.7	43.2 / 46.1	64.7	17.3 / 22.1	38.1	8.7 / 10.0

Stormwater *E. coli* (CFU $100ml^{-1}$)

Site	Dry Weather 1 (5/20/12)	95% CI (-/+)	Dry Weather 2 (6/27/12)	95% CI (-/+)	Wet Weather 1 (5/30/12)	95% CI (-/+)	Wet Weather 2 (8/28/12)	95% CI (-/+)	Wet Weather 3 (9/18/12)	95% CI (-/+)
1	414.00	120.9 / 147.2	20.00	17.5 / 35.9	8297.0	2058.0 / 2789.8	228.0	79.2 / 113.7	331.0	95.0 / 118.2
2	2224.00	638.8 / 808.5	9804.00	3197.9 / 4297.6	3257.0	553.9 / 625.3	2382.0	730.2 / 1025.8	7270.0	2513.3 / 3218.7
3	246.00	85.3 / 110.9	74.00	45.0 / 63.3	24196.0	8416 / 21734	48392.0	15792.0 / 45928.0	10950.0	3900.1 / 4140.3
4[a]	[a]		[a]	[a]	24196.0	8416 / 21734	[a]	[a]	[a]	[a]
5	496.00	142.1 / 181.9	2392.00	452.9 / 512.3	8297.0	2058.0 / 2789.8	1956.0	676.2 / 972.7	48392.0	15784.0 / 45930.0
6	10462.00	3412.1 / 46828.3	31.00	24.8 / 42.3	2723.0	888.1 / 1105.9	285.0	87.5 / 107.4	3255.0	1188.8 / 1752.8
7	20.00	17.5 / 35.9	754.00	142.8 / 161.9	145.0	58.9 / 89.0	158.0	63.9 / 93.0	379.0	101.2 / 116.4
8	20.00	17.5 / 35.10	134.00	56.8 / 83.6	2105.0	563.1 / 704.7	155.0	59.7 / 85.5	341.0	104.3 / 138.7
9	10.00	10.0 / 27.0	884.00	185.0 / 210.0	4106.0	1499.8 / 2083.2	512.0	107.4 / 123.9	1223.0	351.2 / 449.3
10[b]	[b]	[b]	[b]	[b]	3654.0	1334.6 / 1900.5	906.0	259.9 / 334.5	128.0	59.0 / 89.4
11	959.00	256.9 / 316.6	31.00	24.8 / 42.3	24196.0	16303.7 /	570.0	130.2 / 152.9	4884.0	1783.6 / 2330.5
12	1130.00	236.4 / 264.6	1019.00	292.3 / 384.8	8279.0	8416 / 21734	8164.0	2662.8 / 3581.9	48392.0	15784.0 / 45930.0
13	959.00	256.9 / 316.6	359.00	110.3 / 144.4	10462.0	3412.1 / 4628.3	1576.0	422.0 / 547.1	12033.0	3924.6 / 5474.0
14	246.00	85.3 / 110.9	31.00	24.8 / 42.3	3441.0	987.9 / 1283.5	988.0	283.6 / 365.4	1050.0	199.2 / 225.6

[a] Samples not collected from site 4 for this study as it is the end of a drainage pipe with no associated sediment
[b] Samples not collected at site 10 during dry conditions as there is no permanent water present

Table 2. Stormwater and sediment *E. coli* profile data. *E. coli* concentrations in sediment and stormwater for profiled rain events with associated measurement error.

Sediment *E. coli* (MPN g^{-1})

	Profile 1						Profile 2					
Site	Pre Rain (8/27/12)	95% CI (-/+)	During Rain (8/28/12)	95% CI (-/+)	Post Rain (8/31/12)	95% CI (-/+)	Pre Rain (9/17/12)	95% CI (-/+)	During Rain (9/18/12)	95% CI (-/+)	Post Rain (9/19/12)	95% CI (-/+)
1	243.2	84.1 / 115.9	57.8	16.6 / 21.5	715.3	233.3 / 678.9	184.0	67.2 / 93.3	7.7	2.5 / 3.3	20.1	4.9 / 5.8
2	15.2	4.4 / 5.6	679.2	221.5 / 644.6	612.5	199.8 / 581.4	70.1	21.5 / 28.5	48.7	8.3 / 9.6	100.3	51.8 / 79.9
3	374.7	100.3 / 115.7	53.3	11.1 / 12.1	420.7	145.5 / 193.2	23.2	5.3 / 6.6	16.5	4.7 / 6.0	31.2	6.4 / 7.3
4[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]
5	24.9	6.7 / 8.2	53.8	16.5 / 23.6	76.0	27.8 / 40.4	25.1	7.2 / 9.0	9.4	2.5 / 3.3	18.9	3.3 / 3.9
6	324.8	106.0 / 142.5	413.1	142.8 / 189.7	33.9	6.4 / 7.3	29.5	5.6 / 6.3	19.8	4.9 / 5.8	37.9	8.7 / 10.0
7	70.7	17.5 / 20.8	7.5	2.6 / 3.5	89.0	25.6 / 28.5	1.8	1.3 / 1.7	16.4	4.7 / 6.0	47.4	8.3 / 9.6
8	29.4	7.3 / 8.7	30.7	8.2 / 9.4	9.8	3.0 / 3.9	12.1	3.2 / 3.8	7.2	2.5 / 3.2	13.6	3.6 / 4.1
9	40.5	10.8 / 12.4	18.2	4.5 / 5.1	13.9	3.4 / 3.9	2.8	1.5 / 2.3	5.2	2.5 / 3.3	9.8	2.9 / 3.7
10	[b]	[b]	298.9	97.5 / 130.8	15.8	3.3 / 3.9	42.9	11.5 / 14.1	28.4	7.7 / 10.1	62.3	17.1 / 19.3
11	24.7	7.1 / 9.1	78.4	28.6 / 41.6	30.5	5.8 / 6.6	211.4	69.0 / 92.5	100.5	36.7 / 51.0	200.6	68.6 / 98.3
12	643.5	209.9 / 510.7	56.2	11.7 / 13.3	588.3	226.3 / 389.1	67.8	23.5 / 35.6	29.6	5.6 / 6.4	57.8	36.7 / 56.2
13	152.5	52.7 / 71.6	100.4	30.8 / 40.6	272.4	88.8 / 119.5	37.7	9.3 / 11.7	91.5	28.0 / 37.0	195.6	62.4 / 87.6
14	203.6	62.4 / 84.8	64.7	17.3 / 22.1	85.8	21.3 / 24.1	28.9	7.7 / 9.4	38.1	8.7 / 10.0	74.9	27.8 / 40.4

Stormwater *E. coli* (CFU100ml^{-1})

	Profile 1						Profile 2					
Site	Pre Rain (8/27/12)	95% CI (-/+)	During Rain (8/28/12)	95% CI (-/+)	Post Rain (8/31/12)	95% CI (-/+)	Pre Rain (9/17/12)	95% CI (-/+)	During Rain (9/18/12)	95% CI (-/+)	Post Rain (9/19/12)	95% CI (-/+)
1	81.6	44.5 / 71.8	228.0	79.2 / 113.7	1376.0	395.0 / 546.5	63.0	14.0 / 17.0	331.0	95.0 / 118.2	318.0	91.7 / 109.5
2	517.2	108.2 / 124.8	2382.0	730.2 / 1025.8	1071.0	286.4 / 355.6	2603.0	849.4 / 1048.9	7270.0	2513.3 / 3218.7	459.0	114.2 / 131.2
3	2419.6	789.2 / 2296.5	48392.0	15792.0 / 45928.0	39726.0	15286.0 / 26274.0	341.0	104.3 / 138.7	10950.0	3900.1 / 4140.3	4978.0	942.0 / 1090.0
4[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]
5	547.5	189.5 / 256.5	1956.0	676.2 / 972.7	884.0	185.0 / 210.0	754.0	216.4 / 271.8	48392.0	15784.0 / 45930.0	1296.0	372.0 / 472.5
6	131.4	25.4 / 28.6	285.0	87.5 / 107.4	473.0	108.0 / 124.0	97.0	52.5 / 74.6	3255.0	1188.6 / 1725.8	275.0	95.4 / 117.4
7	28.1	10.1 / 12.9	158.0	63.9 / 93.0	341.0	104.3 / 138.7	20.0	17.5 / 35.9	379.0	101.2 / 116.4	323.0	99.2 / 120.5
8	38.8	11.1 / 14.0	155.0	59.7 / 85.5	103.0	51.8 / 79.9	51.0	34.7 / 54.5	341.0	104.3 / 138.7	52.0	35.6 / 53.4
9	22.0	7.2 / 10.2	512.0	107.4 / 123.9	75.0	44.9 / 62.1	0.0	0.0 / 0.0	1223.0	351.2 / 449.3	86.0	49.3 / 56.8
10	[b]	[b]	906.0	259.9 / 334.5	146.0	59.1 / 88.0	[b]	[b]	128.0	59.0 / 89.4	[b]	[b]
11	488.4	178.4 / 233.6	570.0	130.2 / 152.9	462.0	105.4 / 123.6	5172.0		4884.0	1783.6 / 2330.5	618.0	128.9 / 140.8
12	2419.6	789.2 / 2296.5	8164.0	2662.8 / 3581.9	8164.0	2662.8 / 3581.9	3784.0	1160.0 / 1477.5	48392.0	15784.0 / 45930.0	823.0	220.3 / 268.0
13	365.9	133.9 / 189.1	1576.0	422.0 / 547.1	373.0	99.9 / 122.4	272.0	94.3 / 125.0	12033.0	3924.6 / 5474.0	238.0	82.3 / 106.7
14	76.3	46.3 / 60.7	988.0	283.6 / 365.4	246.0	85.3 / 110.9	328.0	93.9 / 114.0	1050.0	199.2 / 225.6	74.0	44.7 / 53.1

[a] Samples not collected from site 4 for this study as it is the end of the drainage pipe with no associated sediment
[b] Pre Rain samples not collected from site 10 as it was totally dry prior to rainfall

Table 3 - Grain size analysis results with and without hydrogen peroxide treatment provided. Sediment grain size characteristics in lower panel reported using phi, a log base 2 scale.

Grain Size with and without Peroxide Treatment

Site	Median Grain Size w/o H_20_2 Treatment (μm)	Median Grain Size with 48hr H_20_2 Treatment (μm)
1	194.0	211.1
2	194.0	387.5
3	5.6	19.1
4	[a]	[a]
5	282.1	355.1
6	213.2	224.8
7	8.9	9.3
8	194.2	29.9
9	6.2	39.7
10	194.2	179.3
11	716.8	495.5
12	282.1	37.4
13	5.6	117.4
14	18.9	7.8

Sediment Sample Characteristics and Organic Content

Site	Mean (phi)	Median (phi)	Standard Deviation (phi)	Skewness (phi)	Kurtosis (phi)	Organic Content (%)
1	2.24	2.17	2.06	0.22	3.14	2.3
2	1.37	1.38	0.76	-0.02	1.03	0.4
3	5.71	6.10	2.77	-0.11	0.67	4.9
4	[a]	[a]	[a]	[a]	[a]	a
5	1.49	1.52	0.67	-0.04	0.99	0.1
6	2.15	2.16	0.93	0.12	2.45	3.5
7	6.75	6.89	2.14	-0.09	1.02	9.7
8	5.07	4.70	2.62	0.24	0.67	6.1
9	4.65	4.84	3.18	-0.02	0.66	5.4
10	2.42	2.18	1.73	-0.06	0.86	5.3
11	1.01	1.12	1.07	-0.12	0.85	0.8
12	4.74	4.41	2.80	0.21	0.77	19.2
13	3.09	1.74	2.88	0.65	0.95	10.4
14	6.99	7.37	2.75	-0.19	0.95	18.6

[a] Grain size and organic content not calculated for site 4 as it is the end of a drainage pipe

Table 4. Sediment *E. coli c*oncentration comparisons with findings of previous studies.

		E. coli (MPN g^{-1})		
Investigator	Environment	Range		Mean
Curtis, Trapp	Stormwater drainage streams (SC)	2.90E+00	2.09E+03	1.66E+02
Beversdorf et al. (2006)	Great Lakes urban beach, sand	1.00E-03	1.00E+03	
Boehm et al. (2009)	CA, FL, MI beach sand	1.26E+01	2.51E+02	1.63E+02
Byappanahalli et al. (2011)	Stream banks (HI)	8.00E+00	1.00E+04	1.62E+01
Haller et al. (2009)	Bottom sediments in Bay of Vidy, Switzerland	1.00E+03	1.00E+05	
Desmarais et al. (2002)	Tidally influenced river sediments (FL)	1.00E+02	3.00E+02	
Fries et al. (2006)	Neuse River Estuary (NC)	1.00E+01	1.00E+03	

Approximate values based on graphs

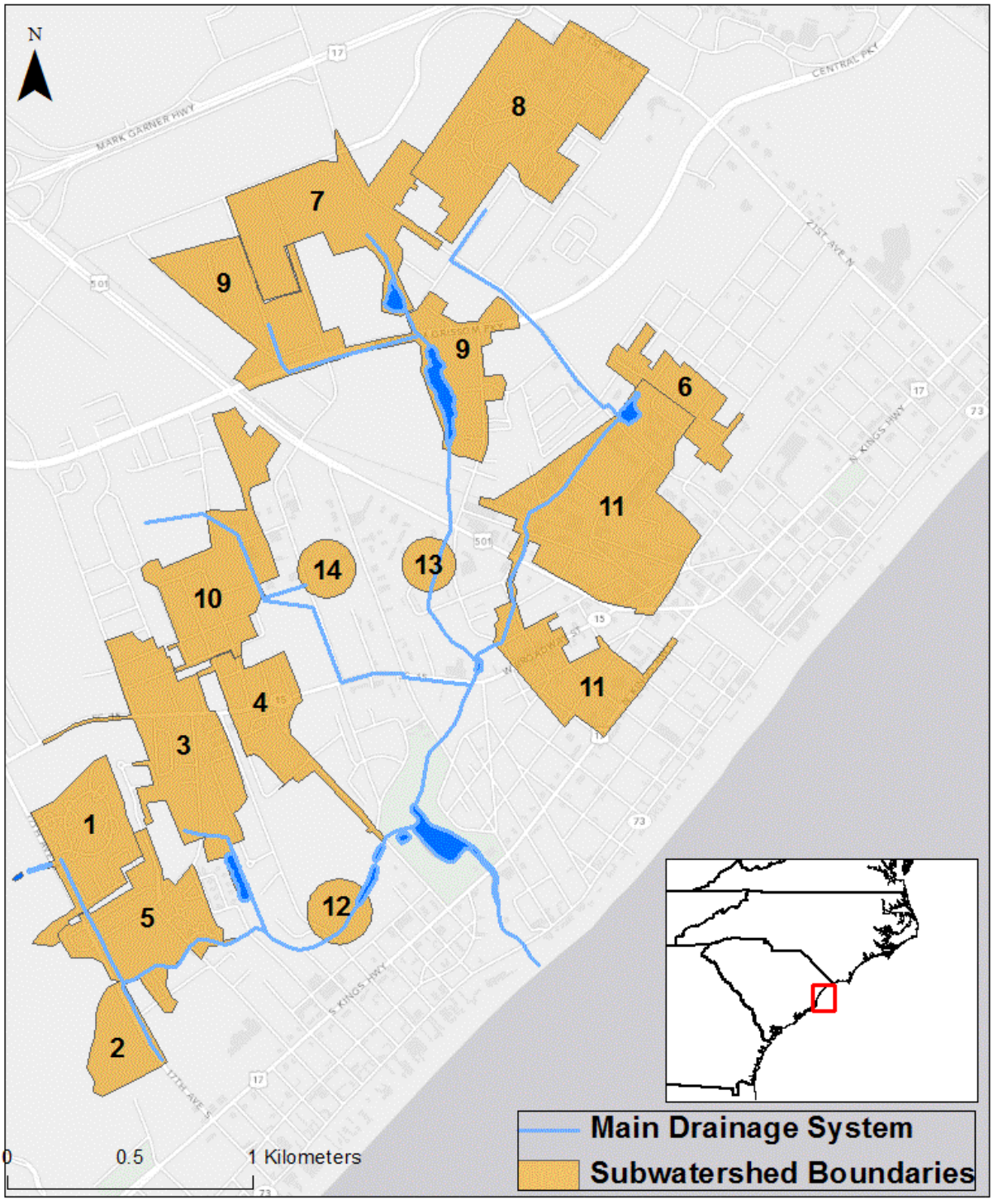

Figure 1 – Withers Swash study area with subwatershed boundaries and hydrologic connections highlighted. Sites 12, 13, and 14 were added as additional sampling locations and do not have defined subwatershed boundaries

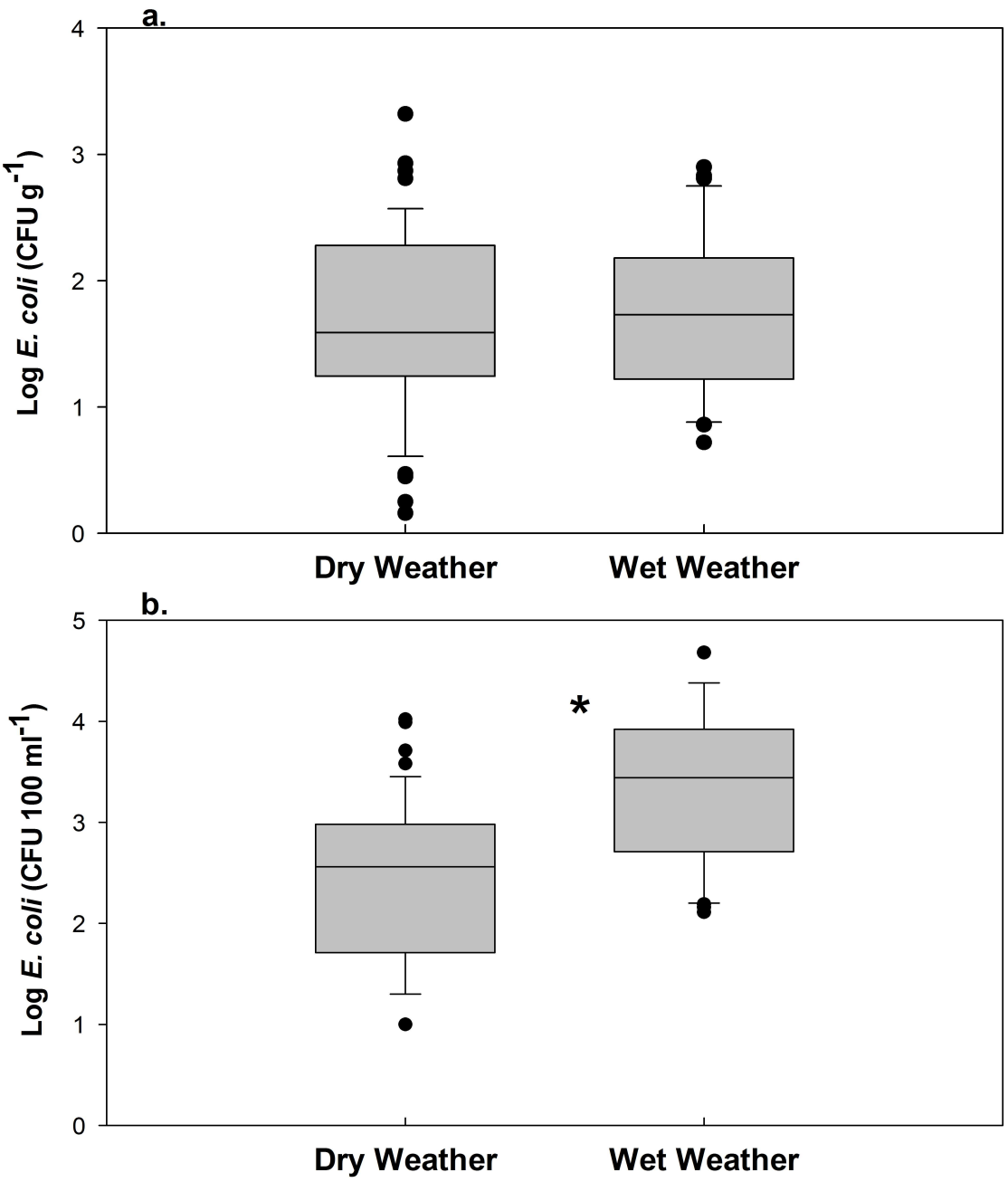

Figure 2 - Field measurements of *E. coli* in sediment (a.) and stormwater (b.) compared based on weather condition at time of sample collection. Asterisks (*) indicate a significant difference between sample groups, $p<0.05$.

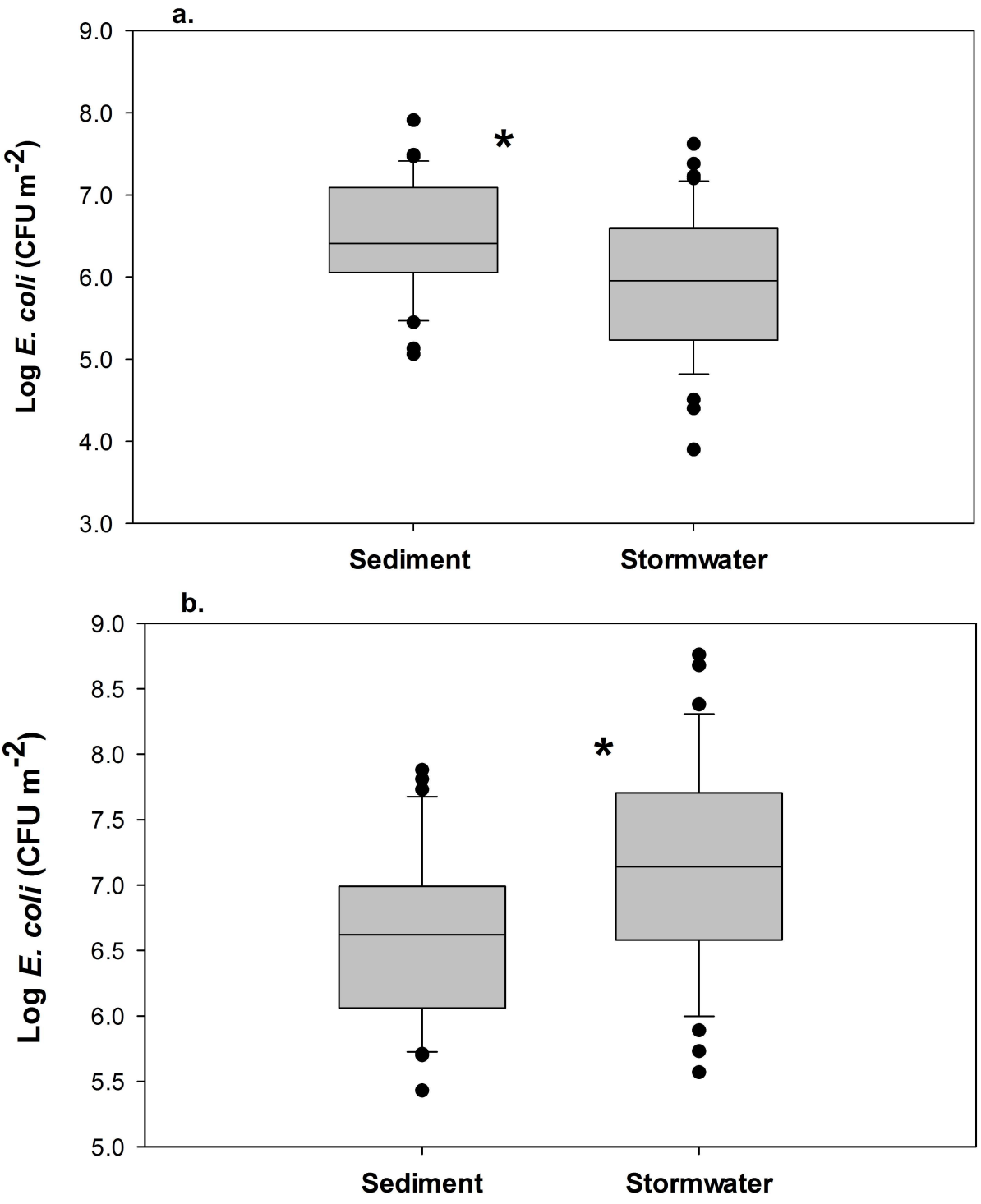

Figure 3 – Field measurements of *E. coli* in sediment and stormwater, normalized to MPN m^{-2}. Concentrations in each matrix compared for samples collected during dry (a.) and wet (b.) weather conditions. Asterisks (*) indicate significant difference between sample groups, $p<0.05$.

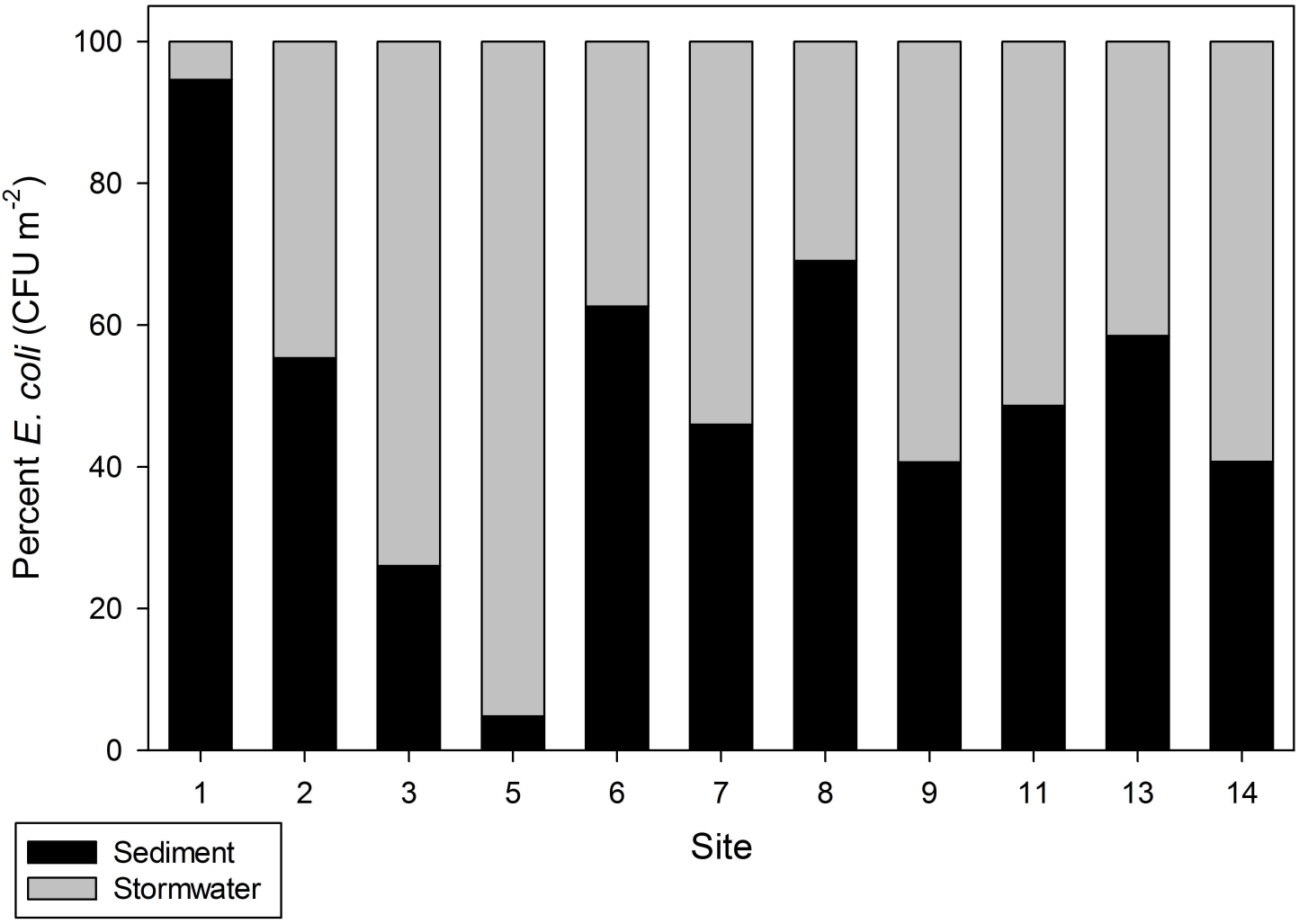

Figure 4 – *E. coli* concentrations (MPN m^{-2}) representing relative sediment and stormwater populations for all sampling events at each site. Site 4 is removed, as it is a drainage pipe with no sediment. Sites 10 and 12 are also removed as site 10 remains dry and site 12 is located within the tidally influenced body of the estuary.

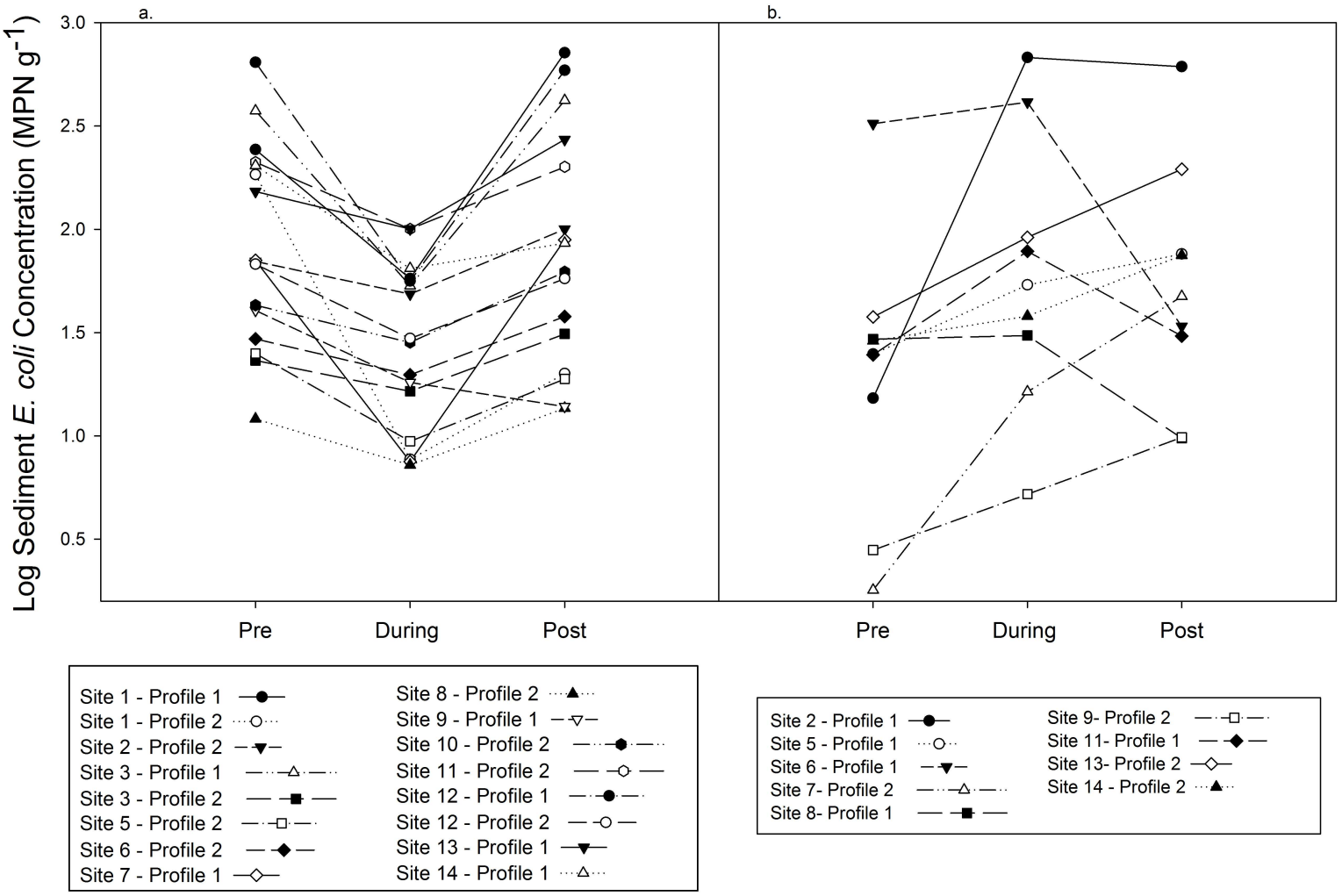

Figure 5 – Rain event profile results showing *E. coli* concentrations in sediment before, during, and after rainfall for profiles that exhibit a bacterial accumulation, flushing, and rebound response (a.) and those that do not (b.). Site 10 has no Pre Rain value, as it does not retain water during dry conditions.

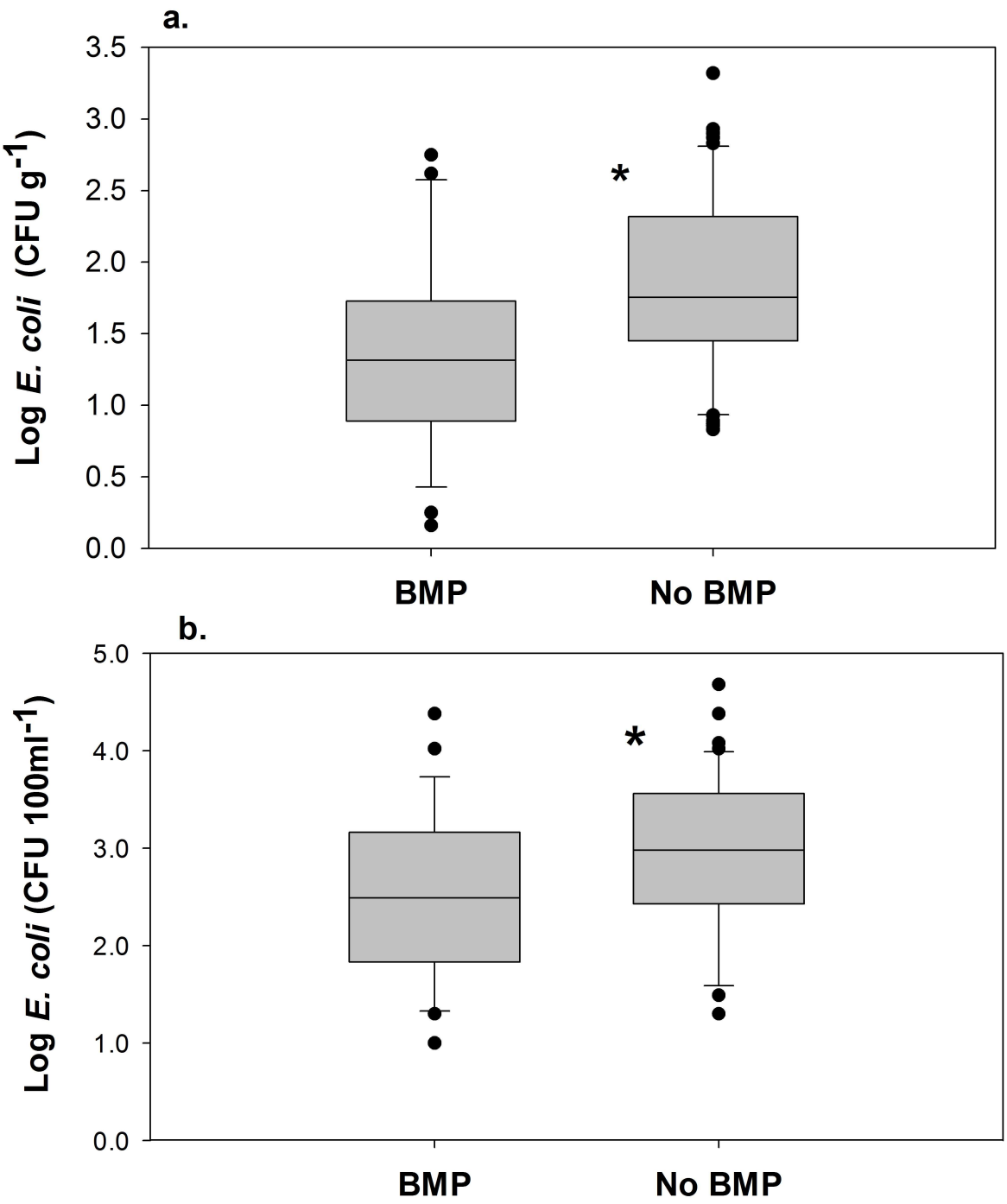

Figure 6 – Effect of best management practice (BMP) stormwater ponds on downstream *E. coli* concentrations in sediment (a.) and stormwater (b.). Asterisks (*) indicate a statistically difference between sample groups, $p<0.05$.

Chapter 3: Examining the colonization and survival of *E. coli* from varying host sources in drainage basin sediments and stormwater

3.1 Introduction

Coastal waterbodies, such as tidal creeks and estuaries, are often the recipients of stormwater runoff accumulated from the larger upstream watershed. Stormwater acts as an important, periodic input of freshwater and nutrients for downstream environments (Wahl et al., 1997). Water entering coastal systems has typically accumulated over a wide area before reaching an estuary or tidal creek as these systems often act as the end-member in a drainage basin. Regular inputs of contaminants may also be generated as stormwater gathers in the drainage basin during storms, transporting chemical and microbial pollutants to receiving waters (Geldrich et al., 1968; Faust, 1976; Ahn et al., 2005). Coastal areas may be disproportionately influencing local watersheds via increased impervious surface due to their often dense human populations. Mallin et al. (2000) described the relationship between upstream development, particularly impervious surface, and the microbial health of downstream tidal creeks, concluding that increasingly dense development yielded degraded water quality.

Fecal indicator bacteria (FIB) are a group of enteric bacteria found commensally in the digestive tract and intestines. FIB serve as a proxy measure of microbial water quality because their presence has been epidemiologically linked to instances of waterborne illness (Wade et al., 2003; Zmirou et al., 2003). It is commonly accepted that these bacteria enter surface waters via overland flow of waste material mobilized by storm events. Research investigating FIB persistence once introduced into a watershed suggested FIB also have the ability to colonize drainage basin sediments where they may experience an extended survival (Craig et al., 2004; Solo-Gabriele et al., 2000; Jamieson et al., 2005; Jeng et al., 2005). These studies observed dense populations of FIB in a variety of aquatic sediments including the banks of alluvial streams (Jamieson et al., 2005), beaches (Boehm et al., 2009), and lakes (Chandran et al., 2011). Previous research in this watershed (Curtis and Trapp, 2014 submitted) as well as studies by

Byappanahalli et al. (2012) and Korajkic et al. (2013) describe enhanced FIB survival in sediments as compared with overlying waters.

Research into the presence of an FIB reservoir in drainage basin sediments also suggests that these sediment-borne bacteria may return to the water column via resuspension due to increased flow associated with storm events (Fries et al., 2008; Walters et al., 2013). It is understood that water quality monitoring comprised of a single grab sample provides limited insight into a dynamic system, particularly during storm events. FIB may be regularly returned to overlying waters due to erosion of upper layer sediments (Jeng et al., 2005; Fries et al., 2006; Boehm et al., 2009). Accumulation and release of FIB could comprise a previously unaccounted for source of bacteria for surface waters. Complications in interpreting water quality data may thus arise as surface water samples use FIB as a proxy for recently added waste material but cannot differentiate between those of recent enteric origin and those from long-term bacterial reservoirs in sediments.

There is a need to better understand the mechanisms affecting the ability of benthic sediments to act as a source and sink for FIB within a watershed. FIB may adsorb to sediment particles (Fries et al., 2006; Friedlander et al., 2013), settle out of suspension (Jeng et al., 2005), and then return to the water column via erosion during increased flow (Solo-Gabriele et al., 2000; Jamieson et al., 2005). Previous studies have not, however, address the possibility that FIB may return to the water column during times of decreased flows as well, despite the fact that *E. coli* are flagellated, motile bacteria (Berg and Tedesco 1975). Grain size and physical characteristics of benthic sediments such as organic content may influence bacterial colonization and resuspension. Jeng et al. (2005) found that FIB preferentially attach to smaller fraction particles (>30 μm), allowing for farther transport and extended suspension. Greater organic content and nutrients associated with smaller grain size particles has also been suggested to enhance bacterial persistence (Chandran et al., 2011; Craig et al., 2004). Field investigations in the Withers Swash watershed showed enhanced persistence may occur via aggregations of smaller grain size sediments with associated nutrients. What is still poorly understood is the capability of these bacteria to enter the water column in the absence of stormwater flow. It is possible that *E.*

coli accumulated in sediments may be regularly remobilized into overlying waters via their own motility or their propensity to adsorb to smaller, more easily eroded particles.

An additional factor that may influence FIB persistence within drainage basin sediments is the host source from which bacteria originate. Research by Korajkic et al. (2013) and Anderson et al. (2005) each found host source to be a significant factor in FIB survival in both water and sediment environments. Both physical make up of waste material and differences in FIB strain found in the gut of various host sources have been attributed to their varying survival once introduced to aquatic and sediment environments. Disparities in survival time once deposited in the environment make the use of FIB as an indicator of recent waste material problematic as typical water quality assessment techniques cannot differentiate between *E. coli* with an extended persistence and that of recent origin. Persisting bacteria are thus a poor indicator of microbial water quality as they may survive in the environment long after the initial deposition of waste material and associated threat of pathogenic contamination.

We investigated key factors affecting FIB survival upon deposition in a watershed using two microcosm experiments. The ability of FIB *E. coli* to enter overlying waters during stagnant conditions was investigated using microcosm environments of local drainage basin sediments (referred to as Water Column Colonization Experiment). This was examined using two grain size treatments to investigate the influence of grain size on FIB persistence and diffusion into the water column. For the Water Column Colonization Experiment it as hypothesized that; *E. coli* originating in sediments would enter overlying waters despite the absence of agitation via flowing water and that smaller grain size microcosm would sustain higher concentrations of *E. coli*. In an additional study (referred to as Host Source Experiment) sterile sediment and ambient stormwater microcosms were spiked with human and avian waste to investigate differences in colonization and survival based on host source. Large and small grain size treatments were used to investigate the importance of physical characteristics as well as host source for FIB survival. It was hypothesized that; host source would significantly effect sediment and water column concentrations of *E. coli*, and that microcosms with smaller grain size sediments would sustain higher concentrations of *E. coli* in sediments.

3.3.2 Methods

3.2.1 Site selection and sediment collection

Sediment samples for each experiment were collected from the same two locations (Sites 6 and 13 **Figure 1**) within the watershed of Withers Swash, an estuary located in northeast South Carolina (**Figure 8**). These sites were selected based on grain size and physical characteristics determined from a previous study conducted in the area (Curtis and Trapp, 2014, submitted). Large grain size microcosms are represented by mineral sediments with a sandy grain size classification (282 µm) and low organic content (3.5%). Small grain size microcosms were established using silty sediments with smaller particle size (5.6 µm) and greater organic content (10.4%).

Grain size of sediments from each site was determined by laser diffraction using a Beckman Coulter LS 13 320 Laser Diffractor. Percent organic content of sediment samples was determined for each site using a loss on ignition (LOI) procedure (ASTM D2974-07a). Sediment for both experiments was collected from each site using a novel extraction method that attempted to maintain natural layering to create a more realistic microcosm environment. Sediment was extracted using an approximately 25 cm length of 20.3 cm diameter PVC pipe sterilized with ethyl alcohol and field rinsed three times prior to use. This pipe was pressed into the sediment to approximately 12.5 cm. Sediment around the microcosm was removed and a round piece of 0.20 cm thick OPTIX® acrylic sheet was placed under the microcosm to aid in sediment removal. A mechanical plug was then placed in the exposed end and tightened to create a vacuum seal for sediment extraction. This pipe, with core intact, was then lifted out of the streambed and placed over an empty microcosm with cap sealed in place. The vacuum seal was then broken by loosening the mechanical plug and the sediment was allowed to slide into the microcosm below. For the Water Column Colonization Experiment three replicate sediment microcosms were collected from each of the two sites using this process. The Host Source Experiment used sediment microcosms collected from the same sites in the described manner.

3.2.2 Microcosm construction

Microcosm environments were created using 20.3 cm diameter PVC pipe, which was cut into approximately 25.5 cm lengths and capped with a PVC cap. Microcosms were filled half with sediment and overlying waters for all experiments (**Figure 1)**. Variables known to effect bacterial survival such as UV exposure and water temperature were controlled in order to isolate the effects of interest. For each experiment microcosms were kept in a covered water bath inside the lab to maintain stable temperatures. Constant ambient temperature was determined using both a HOBO® Water Level Logger and HOBO® Pendant Temperature Data Logger. Throughout the course of each experiment, temperature was found to fluctuate less than 0.5℃ (21.5 ± 0.2 ℃) for Water Column Colonization Experiment and (21.3 ± 0.2℃) for Host Source Experiment.

3.2.3 Experimental design

3.2.3.1 Water Column Colonization Experiment

The Water Column Colonization Experiment focused on the ability of *E. coli* to colonize overlying waters and the effect of grain size on bacterial survival. Sediment cores from a local watershed with intact upper layers were used to represent a realistic drainage basin environment including typical nutrient levels and indigenous biota. As a result the effects of predation and competition on FIB persistence closely reflect ambient watershed conditions. Large and small grain size treatments consisted of three replicate microcosms. Time zero samples of sediment were collected before the addition of water to determine initial bacterial concentrations. Five liters of autoclaved water was then added to the microcosms representing a sterile overlying water column. Negative controls for each grain size treatment were established by autoclaving sediment, adding the same sterile water, and sampling both matrices at each sampling interval. Control microcosms were used to rule out the possibility that bacterial growth in treatment microcosms could be attributed to some effect of the microcosms or water bath environment. Sediment and stormwater were then sampled concurrently as described in section 3.2.4.

3.2.3.2 Host Source Experiment

The Host Source Experiment focused on the differential survival of FIB in stormwater spiked using waste from two common host sources (human and avian), and their ability to colonize sterile sediments. Each host source treatment consisted of two replicate microcosms with sediments from the sites used in the Water Column Colonization Experiment. To examine colonization of sediment by FIB in the water column, sediments were autoclaved in microcosms to kill extant bacteria. Sediment samples were collected from microcosms prior to the addition of stormwater and FIB concentrations in sediment microcosms were determined to be zero. Five liters of stormwater was then added to each microcosm creating an overlying water column. For this experiment ambient stormwater collected the day of well inoculation was used, as it would include any indigenous microbes and more closely represent stormwater conditions present in a watershed at the time of FIB addition via waste material. Controls for each treatment were established by autoclaving sediment, adding sterile water, and sampling both matrices at each sampling interval.

The target final bacterial concentration for spiked microcosms was approximately 10^5 MPN 100 ml^{-1}, a concentration consistent with bacterial levels found in stormwater during rain events within the Withers Swash watershed (Curtis and Trapp, 2014 submitted). Spikes for avian microcosms were created using waste material composited from 5 seagulls. Human source spikes were created using mixed liquor from the Conway wastewater treatment plant (Conway, SC). Waste material for each spike was collected on the day of spike generation and microcosm inoculation. Upon inoculation the water column of each microcosm was manually mixed in order to mimic agitation via stormwater flow at the time of waste deposition. Sediment suspended during the inoculation process was then allowed to settle. Time zero samples of sediment and water were taken to determine initial bacterial concentrations

3.2.4 Microcosm sampling

Sediment and overlying waters were sampled concurrently for all microcosm and control microcosms. Sampling was more intensive at the start of each experiment to obtain better resolution during times more likely to see significant changes in bacterial population. Overlying waters were sampled before sediment in order to avoid resuspending bacteria associated with sediment particles. Water samples were collected and bacterial concentrations determined using Colilert 18 in accordance with manufacturer described guidelines (IDEXX, EPA Method 9223B). The percentage of total water removed due to sampling was calculated (3.3% and 4.0% per well for the Water Column Colonization and Host Source Experiments respectively) and it was determined that this minor fraction of water removed was not likely a source of bias. Sediment samples were collected by scraping approximately 10 g of upper layer sediments using a spoon sterilized with ethyl alcohol and rinsed with Milli Q high purity water before each use. Sediment samples were then split into two aliquots of 5 g each. One aliquot was given a 24 h drying treatment in a 100℃ oven. The resulting dry mass w as used in calculating bacterial concentration. The second 5 g aliquot was added to a 250 ml glass container, which had been washed, sterilized with ethyl alcohol, rinsed with Milli Q high purity water, and allowed to dry. Two hundred milliliters of Milli Q high purity water were added to the container. The container was then manually shaken for period of 2 min to resuspend and desorb *E. coli* bacteria and sediment particles. The mixture was allowed to settle again for a period of 5 min so that larger fraction particles, unlikely to harbor bacteria could again settle out. Optimal shaking and settling times were determined for field investigations and described in section 2.2.3.1. One hundred milliliters of the supernant from this agitation process was then decanted into 100 ml single-use sample containers and analyzed using Colilert for FIB enumeration. Results of sediment bacteria enumeration were reported as MPN per gram of dry sediment.

Total sediment removal for each experiment was determined based on number of samples and used to approximate percent removal of sediment due to sampling. The upper 5 cm of sediment (as opposed to the entire volume of the microcosm) was considered the total sediment volume as this is the section of sediment being sampled and thus potentially impacted by sediment removal. Using estimates of sediment mass for the upper portion of each microcosm

and the number of 10 g samples removed for each experiment it was determined that for the Water Column Colonization Experiment approximately 5.0% of total sediment was removed from small grain size microcosms and 3.9% removed from large grain size microcosms as a result of sampling. For the Host Source Experiment approximately 6.1% of total sediment was removed from small grain size microcosms and 4.8% from small grain size microcosms via sample collection. Thus it was determined that sediment removal due to sampling unlikely biased sediment *E. coli* concentrations.

3.2.5 Decay rate calculation

Bacterial decay was used to investigate survival of FIB in each matrix for both experiments. Decay calculations were based on a standard exponential growth/decay equation.

Formula 1.

$$r = [ln\,(N_t) - ln\,(N_0)\,]/t$$

where;

r = rate of decay

N_t = Log_{10}(MPN 100 ml^{-1}), or Log_{10}(MPN g^{-1}) at time t

N_0 = Log_{10}(MPN 100 ml^{-1}) or Log_{10}(MPN g^{-1}) at time zero

t= time in days

Negative r values represent bacterial population decay while positive r values indicate a growth in total culturable cells. The absolute value of decay rate measurements indicates the magnitude of change in bacterial concentrations.

3.2.6 Statistical analyses

Sediment and overlying waters were sampled concurrently throughout each experiment. As the two matrices were not truly independent, they were analyzed together using multivariate analysis of variance (MANOVA) to examine the effects of independent variables on bacterial concentration in each matrix. All analyses were conducted using SPSS Statistical Software version 20. It was typically necessary to log transform *E. coli* and grain size data in order to meet normality assumptions of parametric statistical techniques. An *a priori* significance level of 0.05 was used for all tests unless otherwise stated.

3.3. Results and discussion

3.3.1 Water Column Colonization Experiment

The Water Column Colonization Experiment investigated the ability of *E. coli* in sediments to colonize overlying waters in the absence of flow (**Figure 9**). Sediment grain size and organic content were examined as independent variables with the potential to influence *E. coli* concentration and persistence. Results indicated grain size characteristics of sediment significantly influenced *E. coli* concentration and persistence. Initial bacterial concentrations of sediment were low for both grain size treatments, with those collected from smaller grain size sites having comparatively higher *E. coli* concentrations at time 0 (34.4 ± 23.5 MPN g^{-1} and 9.9 ± 2.7 MPN g^{-1} respectively, **Table 5**). All samples of sediment and overlying waters collected from control microcosms were below the detection limit of the test. Despite these low concentrations in in the sediment, *E. coli* entered the water column in relatively high numbers, with small grain size microcosms showing greater initial (t_0) bacterial concentration (114.2 MPN $100ml^{-1}$ ± 11.14 SEM) than microcosms with larger grain size sediments (38.1 MPN g^{-1} ± 8.75 SEM, **Table 5**). MANOVA was used to examine grain size effects on bacterial concentration and persistence in each matrix.

Main effects were considered at a significance level of 0.05, while between subject effects required a Bonferroni adjustment, using a 0.025 significance level. The influence of sediment grain size on *E. coli* populations was significant for sediment ($p<0.001$) and overlying waters ($p<0.001$). These findings indicate microcosm microcosms with smaller grain size had significantly greater *E. coli* concentrations in both sediments and overlying waters. As overlying waters were sterile upon starting the experiment these findings suggest an enhanced exchange between the two matrices for smaller grain size microcosms.

While statistical analyses showed a sediment grain size effect on *E. coli* concentrations and persistence, it is believed this effect may be indirect in nature and related to sediment resuspension, not bacterial migration or die off. Water column populations seem to show a two-stage decline. *E. coli* populations in the water column of both small and large grain size microcosms declined more rapidly within the first 96 h (**Figure 9a**). Populations in both treatments declined at a comparable rate for the first 96 h, with each losing approximately 95% of viable bacteria during this time, suggesting their removal is possibly related to settling of smaller fraction sediment particles with adsorbed *E. coli*, not a loss in viable bacteria. Small grain size microcosms had initially higher water column concentrations and a more gradual decline beyond 96 h, possibly due to a greater percentage of small grain size particles remaining in suspension. Large grain size microcosms showed a similar rate of decline for the first 96 h, with overall smaller *E. coli* concentrations throughout. These changes may be attributed to periodic resuspension of small fraction particles associated with sampling. Despite overlying waters being sampled first and care being taken not to agitate sediments, it is possible that smaller particles, of greater bacterial density, were resuspended each time sediment samples were collected. This could have resulted in elevated water column concentrations for the next sampling period, which were more pronounced in smaller grain size microcosms.

MANOVA results examining *E. coli* decay rates (**Figure 11**) support this hypothesis as grain size significantly affected *E. coli* population decay in sediment ($p=0.016$) and overlying waters ($p=0.005$). Enhanced population decay in the water column of large grain size microcosms is likely attributed to the presence of more large grain size particles and thus a more rapid

deposition of sediment and associated bacteria. Conversely, in smaller grain size microcosms decay rates in the water column are slower as there are a greater percentage of small grain size particles, which remain in suspension longer. Results support the hypothesis that grain size affects *E. coli* concentrations and rates of population decay in sediments well as the water column (**Figure 10**). Finding significantly more rapid rate of population decay in large grain size sediments suggests local conditions such as grain size can impact *E. coli* persistence upon entering the sediment environment. Decay rate results showing significantly slower rates of *E. coli* population decay in waters overlying small grain size sediments may be due to long term suspension of small grain size particles and associated bacteria. These findings are of interest as bacterial resuspension has been regularly noted during storm events (Fries et al., 2008; Walters et al., 2013), but exchange with the water column during dry conditions is less understood. It is hypothesized that water column populations were influenced by long term suspension of sediment-adsorbed *E. coli* not a migration from the sediment environment to overlying waters. As a result these findings emphasize the importance of sediments as a long-term means of FIB input to a watershed, even during low flow conditions.

3.3.2 Host source experiment

3.3.2.1 Results

The Host Source Experiment used stormwater spiked with fecal material from two common sources and sediments of two grain size treatments to examine *E. coli* colonization of a sterile sediment environment. Water column bacterial concentrations of spiked microcosms were between 10^5 and 10^6 MPN 100 ml^{-1} at sample time zero **(Figure 13).** Water column populations of bacteria persisted between 24 and 72 h from the time of inoculation (**Figure 13**). Sediment *E. coli* concentrations at time zero ranged from 222.8 – 3239.6 MPN g^{-1}, indicating that FIB added to the overlying water column in the form of spikes colonized the sediment environment within the 1 h elapsed between inoculation and time zero sample collection (**Figure 12**). *E. coli* that colonized

the sediment environment persisted for 480 h in three of the four microcosms, with measurable concentrations still detected at this last sampling interval. All samples of control microcosms for sediment and the water column were below the detection limit of the test.

3.3.2.2 Grain size effects

For this experiment grain size was found be less influential in determining *E. coli* concentrations in sediments and overlying waters. MANOVA results indicated no overall grain size effect (p=0.11) on *E. coli* concentrations for sediments or the water column (**Figure 15**). *E. coli* deposited in stormwater colonized sediments at concentrations ranging from $10^2 – 10^3$ MPN g^{-1} which did not follow any apparent grain size or host source trend (**Figure 12**), suggesting no environmental advantage for small grain size sediments to initial colonization. These results suggest sediment characteristics may have little impact on initial *E. coli* colonization. Water column concentrations did exhibit a grain size trend within host source as large grain size microcosms for human and avian spikes showed a more rapid decay (**Figure 13**). This trend was however not significant (p=0.11) and was obscured by host source effects, which seemed to be more important in determining population decay (**Figure 13**). Examining *E. coli* population decay via MANOVA showed smaller grain size microcosms with significantly reduced population decay rates in the water column ($p<0.001$), but not in sediments (p=0.255). These results helped confirm the importance of bacterial adsorption and resuspension. As suggested in the Water Column Colonization Experiment, diminished decay rates for the water column in small grain size microcosms are possibly the result of mobilization of smaller fraction particles and the associated *E. coli* during sampling. Small grain size particles remain in suspension longer and likely contribute to bacteria values observed in water column samples. These results are consistent with the findings of previous studies (Solo-Gabriele et al., 2000; Jeng et al., 2005, Jamieson et al., 2005) as well as field research (**Section 2.5**) in this watershed which provide evidence for bacterial contributions to overlying waters via sediment resuspension. Concentrations of spikes were made intentionally similar therefore it is not surprising that early measures of water column concentrations are not significantly different despite differences in grain size. As a result there

was no detectable grain size effect on bacterial concentration. Water column population decay, or more likely bacterial remobilization, is however significantly affected by grain size as this directly influences resuspension. Smaller grain size microcosms showed diminished rates of population decay for the water column in both the Host Source and Water Column Colonization Experiments, suggesting a grain size influence on water column bacteria concentrations via long-term suspension of sediment-adsorbed bacteria. These findings corroborate those of the Water Column Colonization Experiment and suggest the possibility of regular bacterial contributions from the sediment environment given minimal agitation.

3.3.2.3 Host source effects

Host source of bacteria was shown to be an important factor in *E. coli* concentration and persistence. MANOVA found host source significantly impacted concentrations of *E. coli* in sediments ($p<0.001$) but not the overlying water column ($p=0.138$) (**Figure 14**). These results were significant for sediment concentrations regardless of grain size, with human source spiked microcosm microcosms sustaining about ten times higher concentrations of *E. coli* (**Figure 14a**) than those spiked with avian waste (means of 2877.2 and 248.5 MPN g^{-1} respectively). As sediments were sterile prior to stormwater addition these results suggest the importance of host source, rather than grain size, to initial bacterial colonization. In this experiment using sterilized sediments, host source significantly influenced sediment *E. coli* concentration. Conversely The Water Column Colonization Experiment used ambient sediments and found microcosms with smaller grain size sediments associated with larger *E. coli* concentration and diminished decay. Comparing the findings of these two experiments suggests that once established, long-term persistence may be governed by environmental factors such as grain size and organic content. This notion is consistent with previous studies suggesting the advantages of adsorption to smaller grain size particles, which include access to sediment-bound nutrients (Davies et al., 1995; Haller et al., 2009), protection from protozoan predation (Davies and Bavor, 2000), and a potential shelter from UV inactivation. Water column differences in FIB concentration followed a similar

pattern, with populations in avian spiked microcosms dying off before human spiked microcosms regardless of grain size (**Figure 13**). This trend but did not reach statistical significance, which may be due in part to the limited survival of FIB in the water column and thus small number of measurements available for analysis.

The effect of host source was apparent in bacterial decay rates as FIB of human origin in each matrix decayed at significantly slower rates than those spiked with avian waste (**Figure 15**). Finding a significant host effect for decay of both sediment and water column populations is possibly due to the fact that sediment concentrations were significantly effected by host source, and sediments may indirectly control water column decay rates via resuspension. Host source influences concentrations in sediments, while grain size may control water column decay rates based erodibility . This trend is apparent in water column decay curves (**Figure 13**) as human spiked microcosms of small grain size had both the highest concentration (due to host source, **Figure 14**) and the longest persistence in the water column (due to small grain size and resuspension). All host source/ grain size combinations followed this pattern (**Figure 15**) with avian spiked large grain size environments dying off most rapidly.

3.3.3 *E. coli* in the environment

3.3.3.1 Evidence for bacterial resuspension

Analysis of sediment and water column *E. coli* concentrations (**Figure 9, Figures 12,13)** and decay rates (**Figure 11, 15**) suggested an exchange with the overlying water column in the absence of flow. Resuspension of sediment-adsorbed bacteria is suggested, as overlying waters in the Water Column Colonization Experiment were sterile prior to their addition to microcosm microcosms, but maintained *E. coli* concentrations for up to 240 h (**Figure 9a**). As noted, it was hypothesized that water column concentrations of *E. coli* may be indirectly controlled by grain size via erodibility. Examining water column *E. coli* decay curves for the Water Column

Colonization Experiment suggests initial population decay (0-96 h) may be governed by this mechanism. Calculating estimated sediment particle settling times supported this hypothesis. Using a settling velocity equation proposed by Dietrich (1982) we estimated that particles would require a diameter of approximately 1.0 μm or smaller to remain in suspension for 96 h. In examining the frequency distribution provided by grain size analysis it was determined that about 5.23% of particles for the small grain size treatment are 1.0 μm or smaller. At this size class and below sediment particles would fall less than approximately 11.5 cm in 96 h, a distance smaller than the height of the water column in microcosm microcosms. In large grain size microcosms only 0.84% of particles fall below 1.0 μm. While smaller grain size microcosms showed greater *E. coli* concentrations, the similarity in shape of their decay curves suggests they are controlled by a similar mechanism, such as the deposition of small fraction particles and associated bacteria. As smaller grain size microcosms have a larger portion of these very fine particles water column populations remain higher even in the absence of flow due to a greater amount of suspended particles and associated bacteria.

Calculating a typical settling rate of freely floating *E. coli* bacteria provided additional evidence for the combination of processes governing FIB mobilization and removal from the water column in the absence of flow. The second stage (96-336 h) of the water column *E. coli* population decay curve for the Water Column Colonization Experiment (**Figure 9a**) showed a more gradual removal of bacteria for each grain size treatment. Here it was hypothesized that a portion of *E. coli*, which have not adsorbed to sediment particles are slowly settling out of suspension. While *E. coli* have a length which is similar to that of sediment particles which were estimated to remain in suspension for approximately 96 h (1 - 2.5 μm) the settling velocity of a sediment particle and a bacterium vary dramatically based on differences in density. Using estimates provided by Liu et al., (2006) it was calculated that an *E. coli* bacterium would settle at a rate of 0.046 cm h^{-1}. At this rate *E. coli* bacteria could fall the 12.25 cm height of the water column in approximately 266 h. These estimates of settling velocity are only an approximation but suggest a small portion of freely floating bacteria which may have remained in suspension for the entirety of the experiment and account for *E. coli* concentrations in the 96-336 h portion of the

water column decay curve. These findings provide evidence for the long-term influence of sediment-bound FIB to water column concentrations during dry conditions as *E. coli* originating in sediments entered and remained in the water column for at least 14 days in the absence of turbulence or flow. Bacterial analysis for this study was unable to determine the relative portions of freely floating and sediment associated bacteria for a given water sample. It is likely that all water column samples include a combination of both. However, as water column *E. coli* concentrations followed the grain size hypothesis it is believed that the primary mechanism is related to sediment adhesion and erosion.

These findings do not suggest that sediments must be approximately 1μm in order to harbor FIB. They do however provide evidence for bacterial adsorption to sediments of this size and suggest their ability to influence water column bacteria concentrations given minimal flow. Microcosm microcosms for these experiments were kept intentionally stable with care being taken to minimize sediment resuspension during sampling. Even given this controlled environment, bacteria originating in sediments entered sterile waters and persisted for 14 days. Additionally, sediment settling times are conservatively estimated based on calculations that simplify complex processes. It is likely that environmental conditions such as the shape of sediment particles, concentration of suspended particles, water temperature, and possibility of flocculation would also influence settling rates. Organic content of sediments is an additional environmental variable that could readily influence both bacterial persistence as well as erobidbility and settling velocity. Previous studies in this watershed (Curtis and Trapp 2014, submitted) have shown a positive correlation between organic content and *E. coli* concentration in sediments. Organic matter has a reduced erodibilty but may then settle considerably slower once in the water column. As organic rich particles may also harbor greater concentrations of bacteria their contribution to overlying water quality could be enhanced.

3.3.3.2 *E. coli* persistence and watershed management

The use of FIB, such as *E. coli*, as an indicator of microbial contamination is only as reliable as the strength of correlation with pathogens of concern. Results presented here show *E. coli* persisting substantially longer in sediments than the overlying water column in each experiment (**Figure 9,12 and 13**). Despite disparities among experiments in initial concentration (10^1-10^2 vs 10^5 MPN g^{-1}) and origin of bacteria (sediment vs water column), both show an extended *E. coli* persistence in sediment environments. This suggests that while factors such as host source and sediment characteristics can influence *E. coli* colonization and survival, sediments sustain *E. coli* for longer periods than overlying waters across a variety of conditions. These findings are consistent with similar studies by Davies et al. (1995), Anderson et al. (2005), and Haller et al. (2009) which find an extended FIB persistence in sediments relative to overlying waters. Evidence of FIB persistence in the environment and exchange with overlying waters poses a significant concern for water quality monitoring. Any FIB persistence impedes the ability of a typical FIB test to detect recent microbial impairment, as their survival outside of the host organism has not been shown to correlate microcosm with the survival of the species for which they are an indicator (Lemarchand and Lebaron, 2003; Harwood et al., 2005; Noble and Fuhrman, 2001). Differences in survival of FIB and species such as *Salmonella, Cryptosporidium,* as well as enteroviruses suggest they may be a poor indicator of microbial impairment. Our results are thus important as they confirm the extended persistence of FIB in sediments and provide evidence for their maintenance in the water column given minimal agitation.

The advent of molecular techniques for identifying host sources of microbial impairment has lead to microbial source tracking (MST) methods which aim to improve watershed management by targeting the most significant contributors to water quality degradation. Differential decay of FIB from varying host sources, as presented in the Host Source Experiment, could confound MST efforts as a rapid decay may result in underestimating the importance of a contamination source within the watershed while an extended persistence may exaggerate its actual contribution (Stewart et al., 2013). The importance of host source upon initial deposition may be due to phenotypic variability of different strains of *E. coli* associated with various animals, leading to more resilient strains which survive more readily outside the host organism (Anderson

et al., 2005). Following an initial competition for limited nutrient resources and die off of less fit varieties of *E. coli,* environmental factors such as grain size may become more critical to population persistence. The importance of host source upon initial sediment colonization is supported by results from the Host Source Experiment (**Figure 14**). Grain size and physical characteristics however may better explain long-term persistence as environmental sediments showed greater *in situ* populations of *E. coli* in small grain size sites upon collection (**Figure 9**). This trend was confirmed throughout the Water Column Colonization Experiment by the significant impact of grain size on sediment *E. coli* concentration and persistence. Thus the use FIB as an indicator of poor water quality may be confounded by the variety of bacterial strains entering the environment from various host organisms as well as the direct and indirect effects of drainage basin sediment grain size. Other studies have suggested differences in FIB survival according to host source upon entering the environment (Anderson et al., 2005; Korajkic et al., 2013). Results of the Host Source Experiment supported these findings, as host source significantly affected FIB concentrations in sediments (**Figure 14**) and rate of FIB decay in both sediment and overlying waters (**Figure 15**). Results of each experiment present evidence for bacterial contributions to overlying waters in times of minimal agitation and suggest the importance of drainage stream sediment particle size in determining sediment contributions to FIB concentrations in overlying waters.

3.4. Conclusions

Many biological, chemical, and physical factors influence the survival of FIB outside the host organism. This study showed that both grain size and host source can have individual and interactive effects, which influence *E. coli* concentration and survival in stormwater drainage basin sediments and overlying waters. Results indicated extended *E. coli* persistence in sediments relative to overlying waters under two host source and sediment characteristic conditions. Water column concentrations show *E. coli* originating in sediments can enter the water column under no flow conditions and suggest the indirect control of water column FIB by sediment grain size,

percent composition of small fraction particles, and erodibility. These findings question the efficacy of FIB, such as *E. coli,* based on their extended environmental persistence relative to pathogenic species, variability in persistence based on grain size and host source, and the inability to distinguish between true contributions of waste material and FIB in samples due to resuspension of sediment bacterial reservoirs.

Table 5 - Water Column Colonization Experiment *E. coli e*numeration results for sediment and water column concentrations with associated error.

	Sediment *E. coli* Concentration (MPN g^{-1})				Water *E. coli* Concentration (MPN 100ml^{-1})			
Time	Large Grain Size Wells	SEM (+/-)	Small Grain Size Wells	SEM (+/-)	Large Grain Size Wells	SEM (+/-)	Small Grain Size Wells	SEM (+/-)
0	10.0	1.6	34.4	13.5	38.1	8.8	114.2	11.1
6	11.1	2.5	35.1	6.7	22.0	7.4	98.8	20.1
12	7.4	1.3	57.0	22.2	26.1	3.8	82.7	12.3
18	6.4	2.4	86.4	27.8	15.7	3.7	92.8	5.9
24	4.0	0.5	46.9	18.3	16.5	3.3	92.5	25.2
36	14.8	2.1	35.8	18.0	11.9	1.6	67.6	15.0
48	8.7	0.2	24.6	3.9	6.9	2.1	57.1	17.2
60	4.6	0.7	46.3	9.7	4.7	0.6	47.1	13.7
72	5.6	0.8	12.7	4.0	1.3	1.1	18.7	9.4
96	12.3	3.8	19.8	6.9	2.0	0.9	5.4	1.1
144	3.9	0.5	12.3	2.5	1.3	1.1	4.7	1.5
240	5.4	1.5	24.8	6.5	0.0	0.0	2.0	0.9
336	4.1	1.4	14.7	3.9	0.3	0.3	0.7	0.5

Standard error of mean (SEM) reported for mean of 3 replicate measures for each grain size treatment at each sample time

Table 6 - Host Source Experiment *E. coli e*numeration results for sediment and water column concentrations with associated error.

Sediment *E. coli* Concentration (MPN g^{-1})

Time	Large Grain Size Well with Human Spike	+	–	Time	Large Grain Size Well Avian Spike	+	–
0	3239.6	1171.6	1314.3	0	222.8	137.3	89.2
6	1555.2	296.3	492.9	6	115.5	102.3	60.0
12	1429.8	278.7	478.5	12	61.3	89.7	31.5
18	726.0	189.3	269.1	18	203.1	84.2	61.3
24	2934.1	1140.6	1190.3	24	23.6	61.0	20.5
36	6020.9	2138.2	2549.1	36	36.5	69.5	28.4
48	2259.0	277.0	635.2	48	78.8	71.3	33.4
60	5361.5	1979.5	2270.4	60	11.8	53.5	10.6
72	607.2	160.2	224.1	72	35.4	51.2	28.2
96	3818.1	788.9	1277.4	96	48.3	59.6	34.1
144	582.4	144.7	215.9	144	23.6	60.6	20.0
192	340.8	91.5	126.5	192	23.6	60.6	20.0
240	321.2	124.8	142.1	240	11.8	53.5	10.6
312	658.1	182.9	256.0	312	23.6	61.0	20.5
384	823.9	206.8	290.1	384	0.0	0.0	0.0
480	427.0	102.6	137.6	480	0.0	0.0	0.0

Time	Small Grain Size Well with Human Spike	+	–	Time	Small Grain Size Well with Avian Spike	+	–
0	584.6	229.3	207.2	0	2070.03	845.7	582.2
6	1400.4	407.0	444.5	6	254.24	155.9	111.2
12	2376.2	835.7	798.8	12	111.32	107.1	62.2
18	415.0	168.6	154.6	18	94.78	162.0	28.7
24	1752.1	504.2	523.4	24	162.47	119.8	84.2
36	1672.9	379.8	468.7	36	111.32	107.1	62.2
48	2203.6	557.2	658.0	48	257.25	158.2	107.6
60	2510.9	1060.8	891.1	60	78.23	85.6	50.9
72	8193.3	3387.8	3060.9	72	94.78	97.8	56.9
96	5462.2	2435.0	2142.2	96	240.70	159.7	101.2
144	8193.3	3387.8	3060.9	144	30.09	77.6	25.5
192	9970.7	2882.9	3165.5	192	60.18	83.9	34.4
240	8701.8	2286.3	2600.3	240	60.18	83.9	34.4
312	2967.1	642.5	775.5	312	76.70	84.1	50.9
384	936.2	295.3	297.7	384	30.10	77.6	25.6
480	155.2	123.3	84.2	480	46.00	89.0	35.4

Water *E. coli* Concentration (MPN $100ml^{-1}$)

Time	Large Grain Size Well with Human Spike	+	–	Time	Large Grain Size Well Avian Spike	+	–
0	28970.0	13347.0	8262.0	0	10810.0	3914.0	3106.0
6	15760.0	5471.0	4220.0	6	3270.0	1497.0	1132.0
12	14830.0	5158.0	4258.0	12	1710.0	1029.0	723.0
18	1970.0	1099.0	798.0	18	410.0	542.0	245.0
24	840.0	694.0	469.0	24	1.0	-1.0	1.0
36	310.0	146.0	155.0	36	1.0		
48	200.0	93.0	68.0	48	1.0		
60	0.0			60	1.0		
72	0.0			72	1.0		
96	0.0			96	1.0		

Time	Small Grain Size Well with Human Spike	+	–	Time	Small Grain Size Well with Avian Spike	+	–
0	36540.0	54989.6	13346.0	0	13960.0	18806.0	4010.0
6	20350.0	26758.7	5442.0	6	3170.0	4617.4	1096.0
12	10140.0	13473.9	2710.0	12	2130.0	3227.4	859.0
18	7850.0	10786.1	2254.0	18	520.0	1065.2	344.0
24	1680.0	2656.2	714.0	24	200.0	285.9	68.0
36	1340.0	2208.7	596.0	36	100.0	163.5	48.0
48	520.0	1182.1	291.0	48	0.0	0.0	1.0
60	720.0	1356.3	430.0	60	0.00	0.0	1.0
72	0.0	0.0	1.0	72			
96	0.0	0	1.0	96			

Error value calculated using manufacturerprovided values at 95% confidence interval.

Table 7. *E. coli* Population Decay Values for Water Column Colonization and Host Source Experiments

Water Column Colonization Experiment

Sediment *E. coli* Decay from Time 0			Water *E. coli* Decay from Time 0		
Time	Large Grain Size Wells	Small Grain Size Wells	Time	Large Grain Size Wells	Small Grain Size Wells
0			0		
6	0.189	0.0250	6	-0.653	-0.124
12	-0.283	0.2672	12	-0.218	-0.141
18	-0.285	0.3088	18	-0.373	-0.060
24	-0.512	0.0844	24	-0.260	-0.046
36	0.106	0.0076	36	-0.256	-0.078
48	-0.031	-0.0494	48	-0.318	-0.079
60	-0.164	0.0324	60	-0.340	-0.083
72	-0.097	-0.1104	72	-0.846	-0.160
96	0.022	-0.0422	96	-0.415	-0.258
144	-0.088	-0.0571	144	-0.423	-0.186
240	-0.030	-0.0096	240	0.000	-0.192
336	-0.035	-0.0195	336	0.000	0.000

Host Source Experiment

Sediment *E. coli* Decay from Time 0

Time	Large Grain Size Well with Human Spike	Large Grain Size Well with Avian Spike	Small Grain Size Well with Human Spike	Small Grain Size Well with Avian Spike
0				
6	-0.381	-0.518	0.514	-1.284
12	-0.213	-0.546	0.397	-0.966
18	-0.273	-0.023	-0.074	-0.690
24	-0.012	-0.537	0.159	-0.406
36	0.049	-0.271	0.102	-0.322
48	-0.023	-0.107	0.094	-0.160
60	0.024	-0.314	0.082	-0.224
72	-0.077	-0.139	0.115	-0.173
96	0.005	-0.083	0.075	-0.083
144	-0.040	-0.089	0.058	-0.135
192	-0.041	-0.054	0.037	-0.062
240	-0.034	-0.056	0.025	-0.044
312	-0.017	-0.038	0.016	-0.040
384	-0.012	0.000	0.005	-0.058
480	-0.014	0.000	-0.017	-0.049

Water *E. coli* Decay from Time 0

Time	Large Grain Size Well with Human Spike	Large Grain Size Well with Avian Spike	Small Grain Size Well with Human Spike	Small Grain Size Well with Avian Spike
0	-0.244	-0.551	-0.229	-0.675
6	-0.136	-0.443	0.740	0.007
12	-0.405	-0.579	0.456	-0.266
18	-0.423		0.153	-0.366
24	-0.389		0.081	-0.337
36	-0.331		-0.009	
48			0.013	

Decay rates calculated using exponential decay equation;

$r = [ln(\log N_t) - ln(\log N_0)]/t$

where;

r = decay rate

N_t = *E. coli* MPN 100 ml^{-1}, or MPN g^{-1} at sample time t

N_0 = *E. coli* MPN 100 ml-1, or MPN g-1 at sample time 0

t = time in days

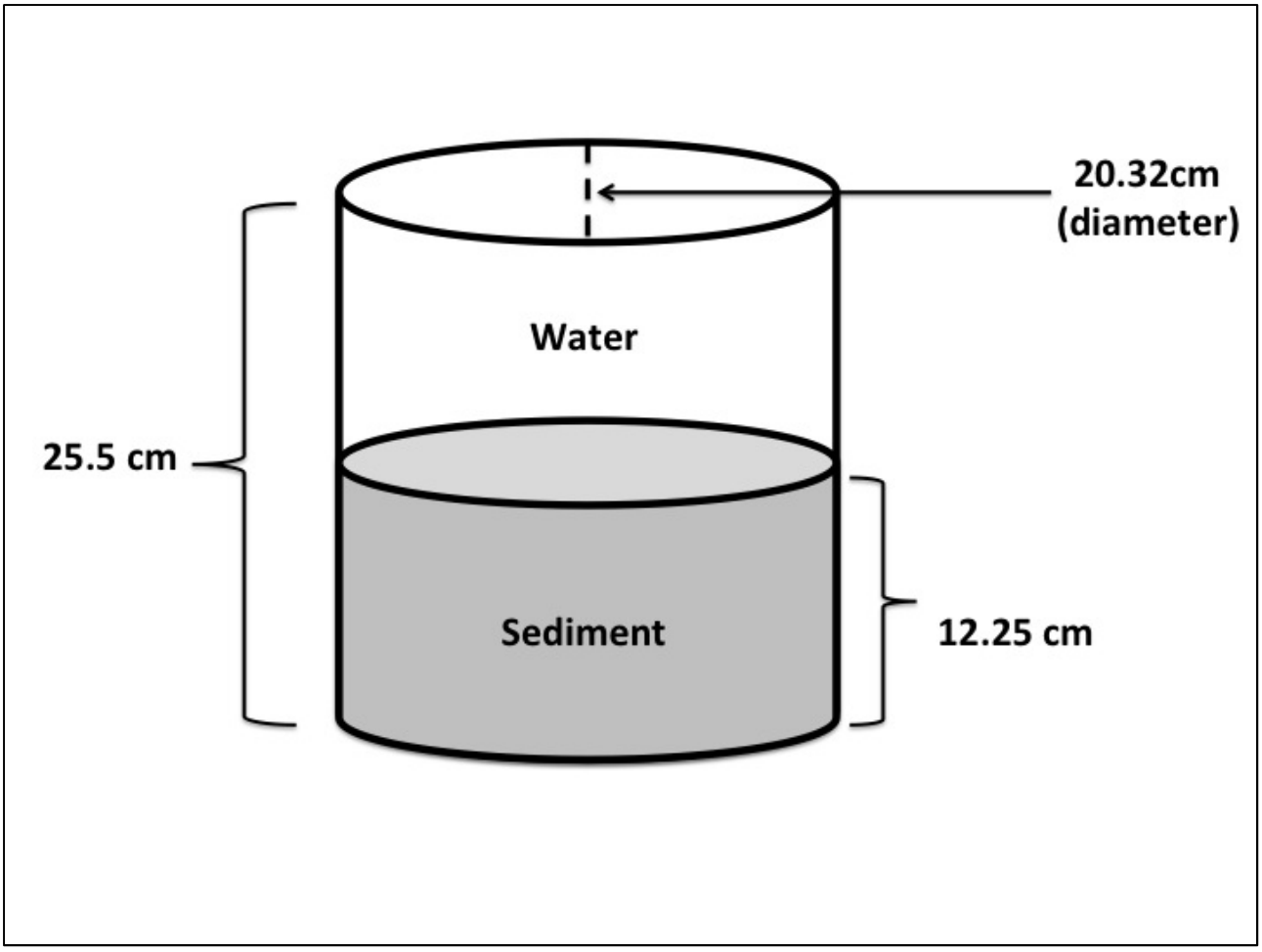

Figure 7– Schematic of microcosm microcosms used for Water Column Colonization and Host Source Experiments.

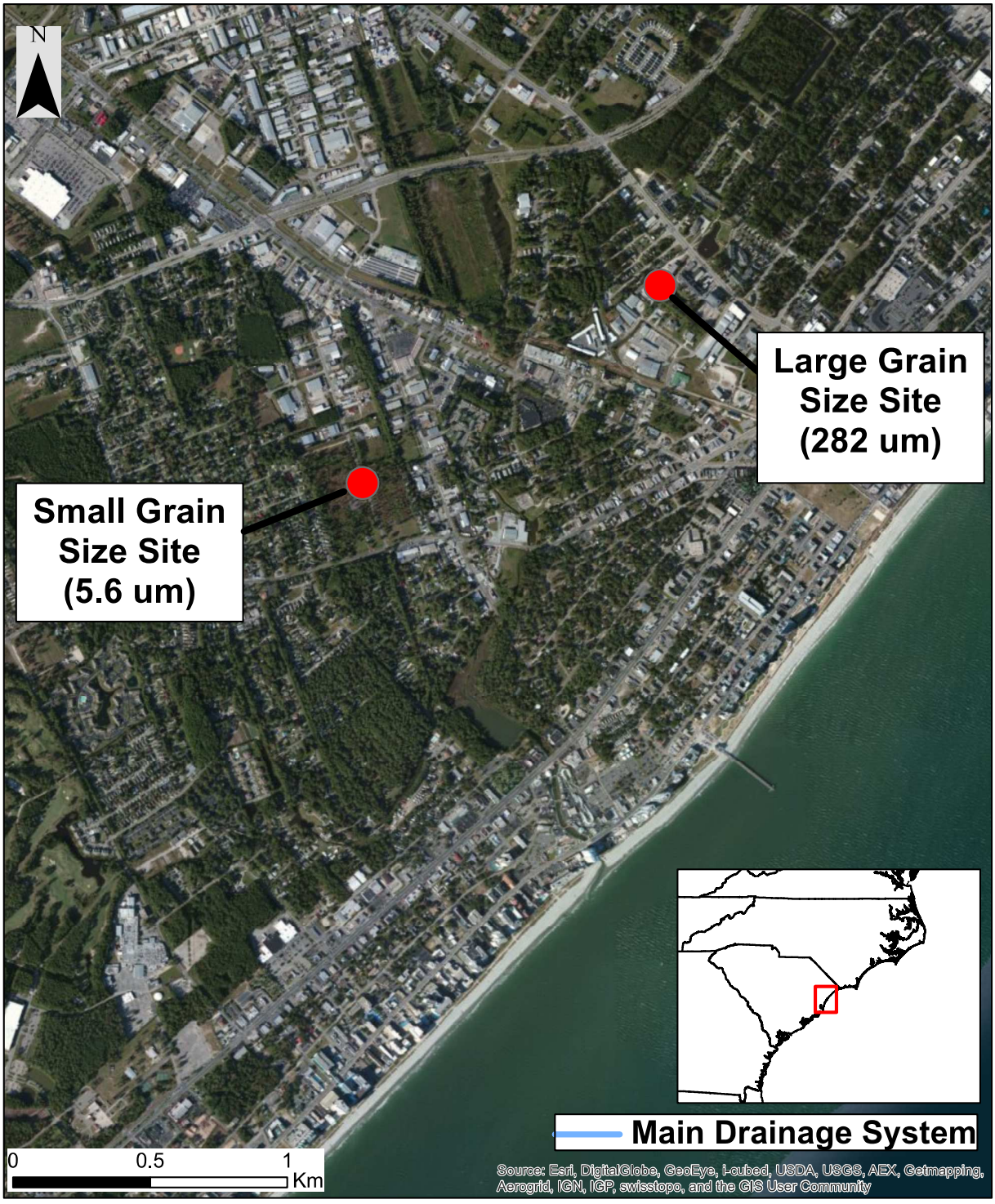

Figure 8 – Microcosm experiment sediment collection location map.

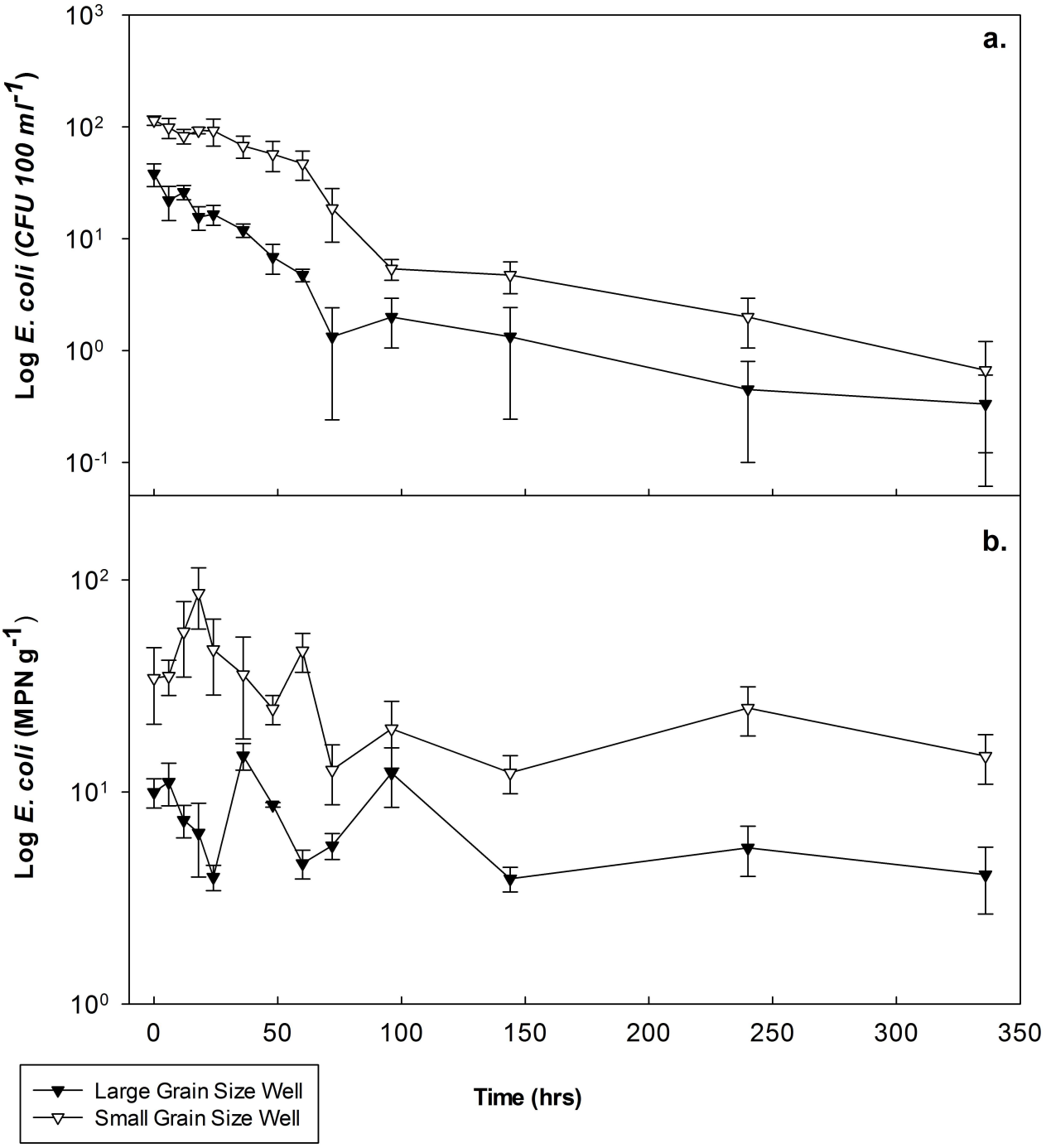

Figure 9 – Water Column Colonization Experiment *E. coli* population decay curves for water column (a.) and sediment (b.) matrices. Error bars represent standard error of replicate microcosms (n=3) for each measurement.

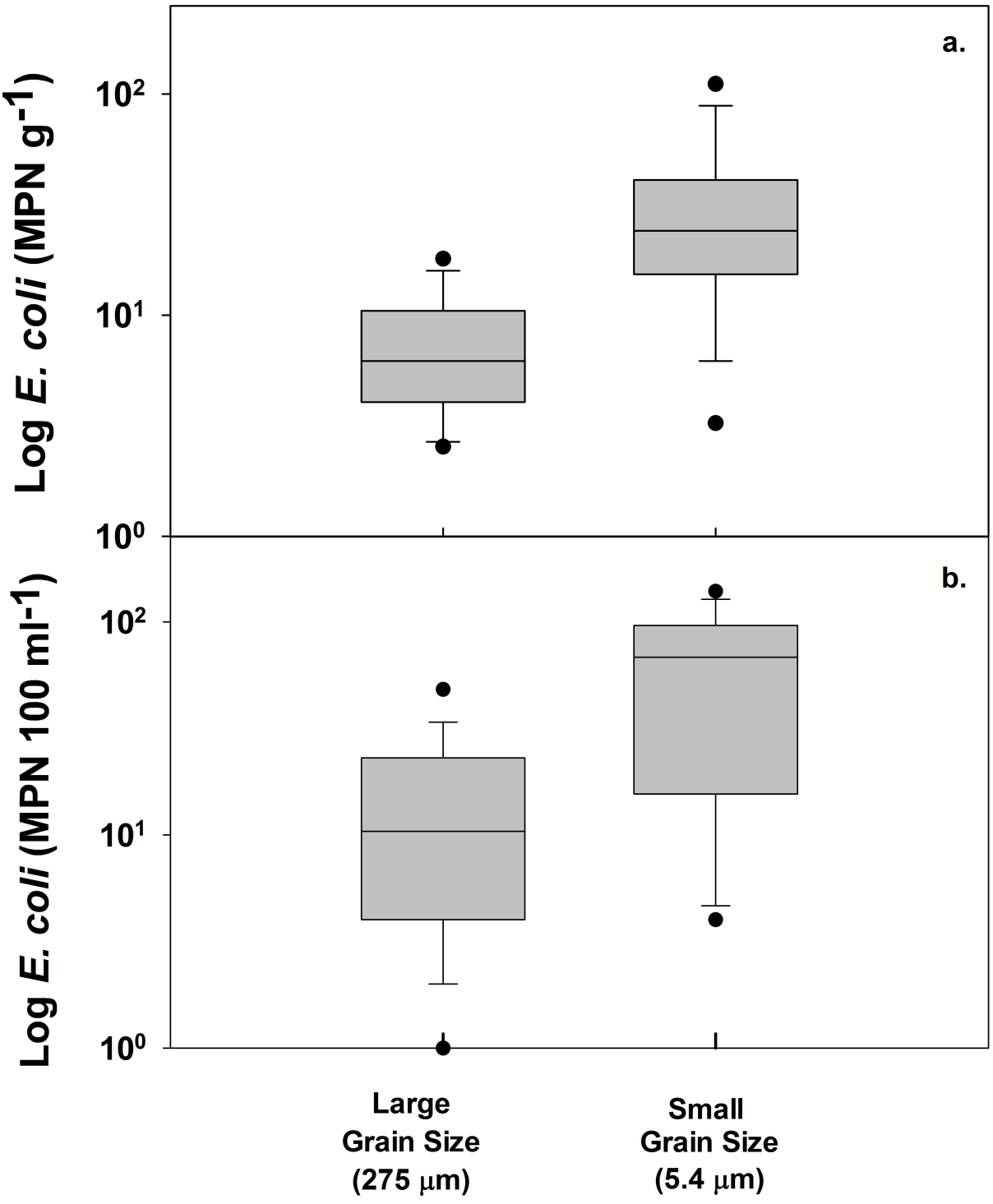

Figure 10 – Water Column Colonization Experiment. Grain size significantly affected *E. coli* concentrations in sediment (a.) and stormwater (b.) matrices.

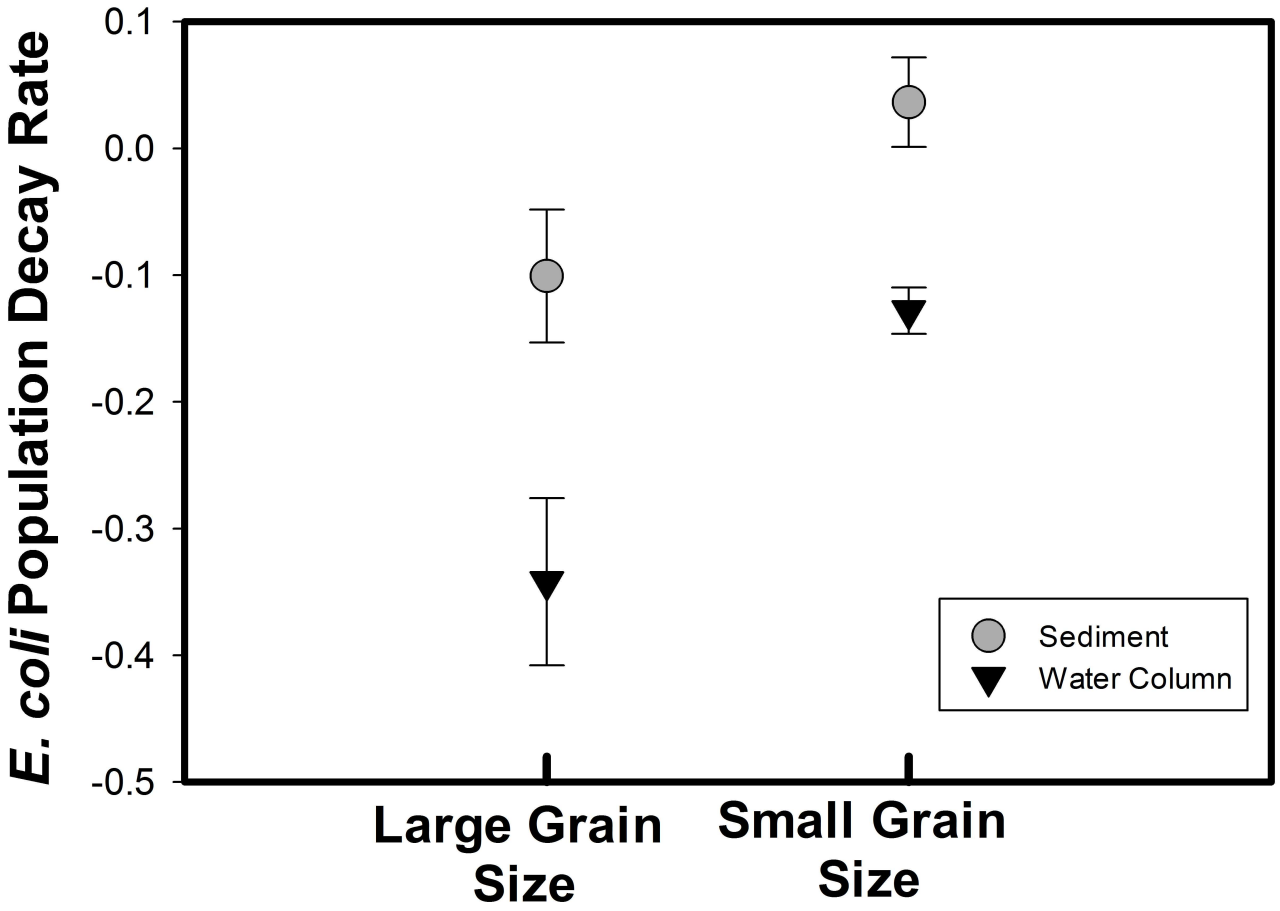

Figure 11 – Water Column Colonization Experiment grain size effects on *E. coli* population decay rate. Mean population decay presented with standard error (n=3) for large and small grain size treatments. Grain size significantly affected *E. coli* concentrations in sediment (p=0.016) and stormwater (p=0.005). Absolute value indicates magnitude of change. Negative values indicate loss in viable bacteria.

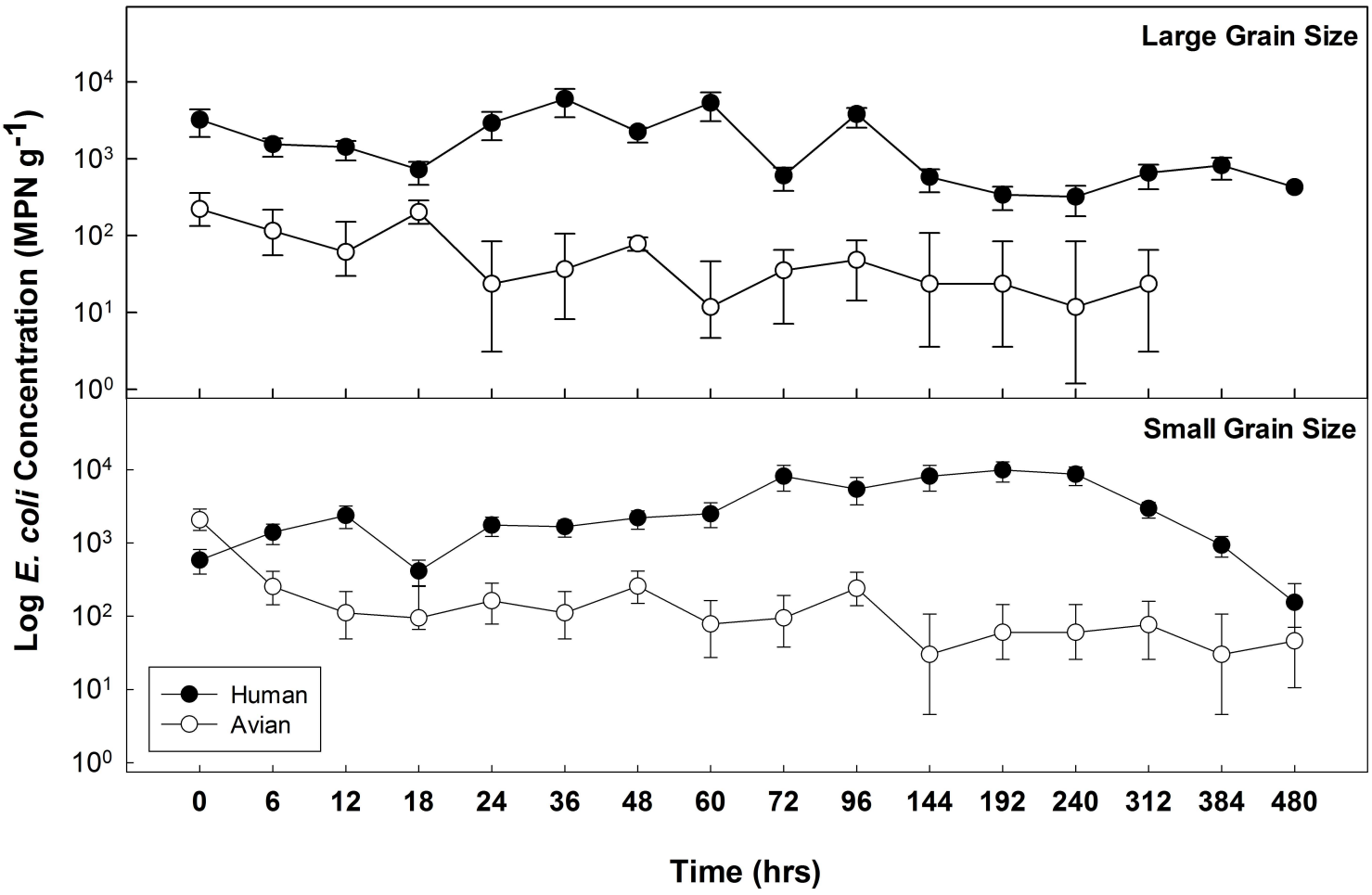

Figure 12 – Host Source Experiment *E. coli* concentrations for each host source (Human, Avian) and grain size (Large, Small) combination. Error bars represent bacterial enumeration method error at a 95% confidence interval.

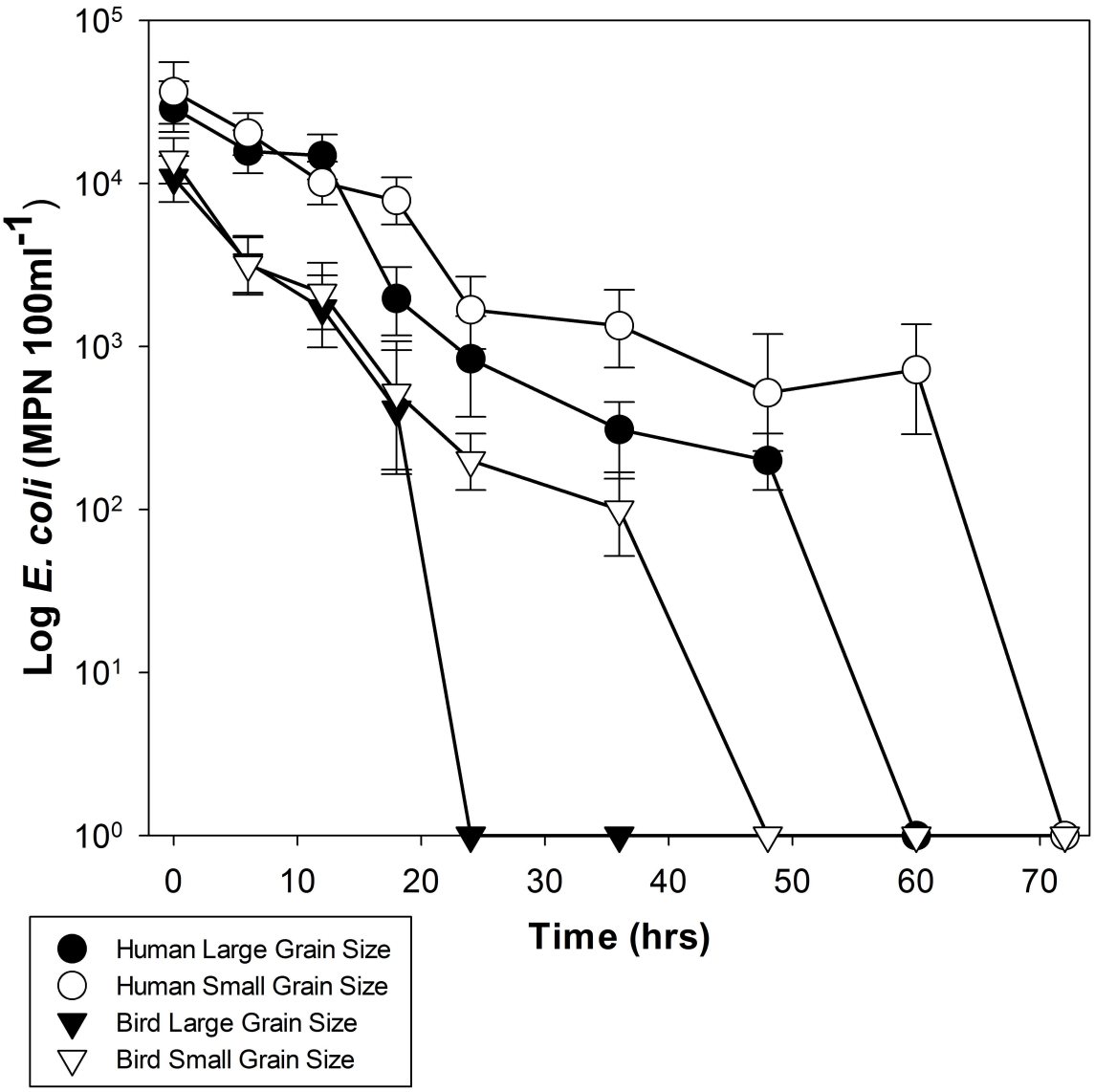

Figure 13 – Host Source Experiment water column *E. coli* concentrations for each host source (Human, Avian) and sediment grain size (Large, Small) treatment combination. Error bars represent bacterial enumeration method error at a 95% confidence interval.

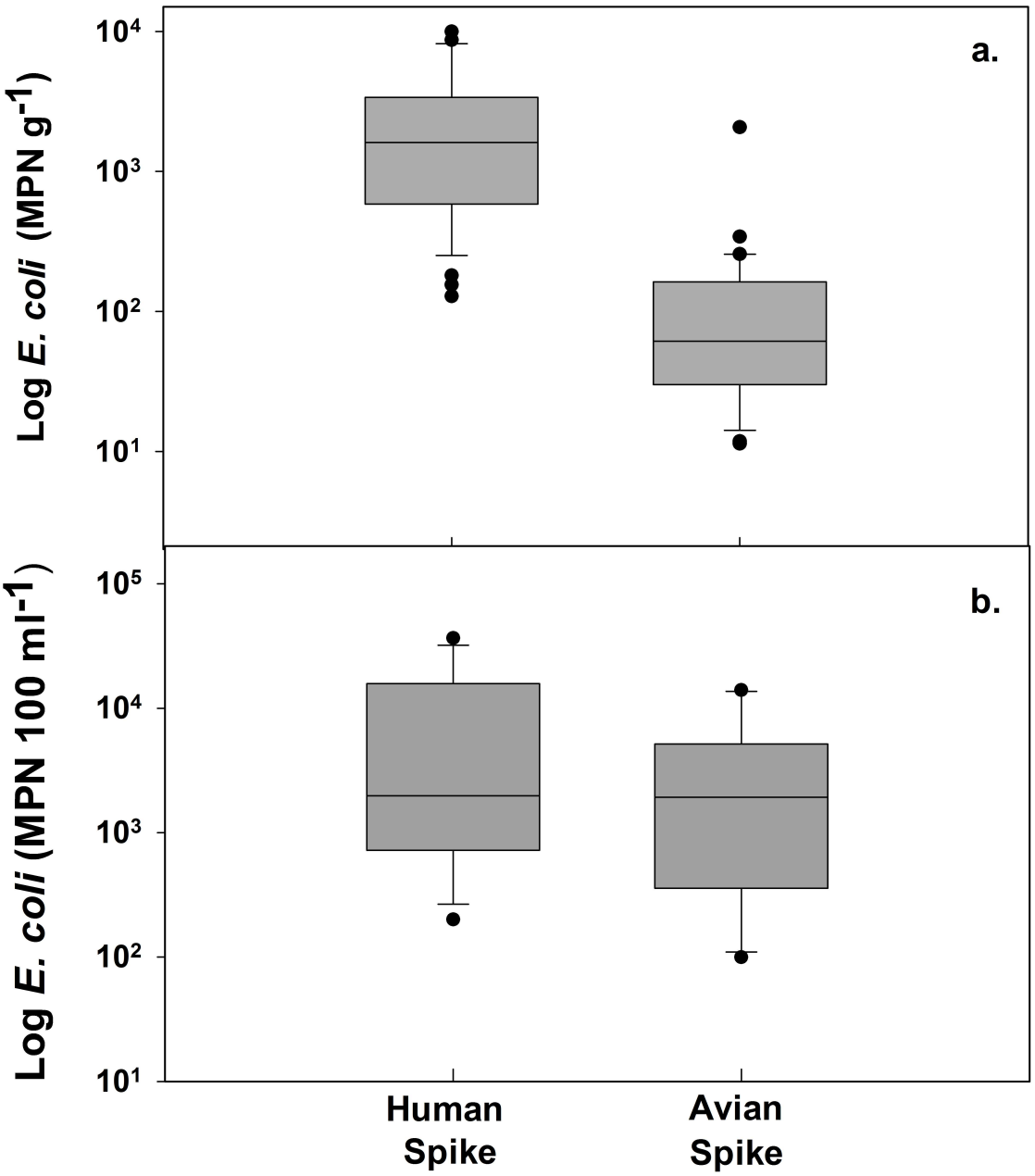

Figure 14 – Host source effect on *E. coli* concentration in sediment (a.) and overlying waters (b.) for Host Source Experiment. Host source significantly affected *E. coli* concentrations in sediment ($p<0.001$) but not the water column ($p=0.136$).

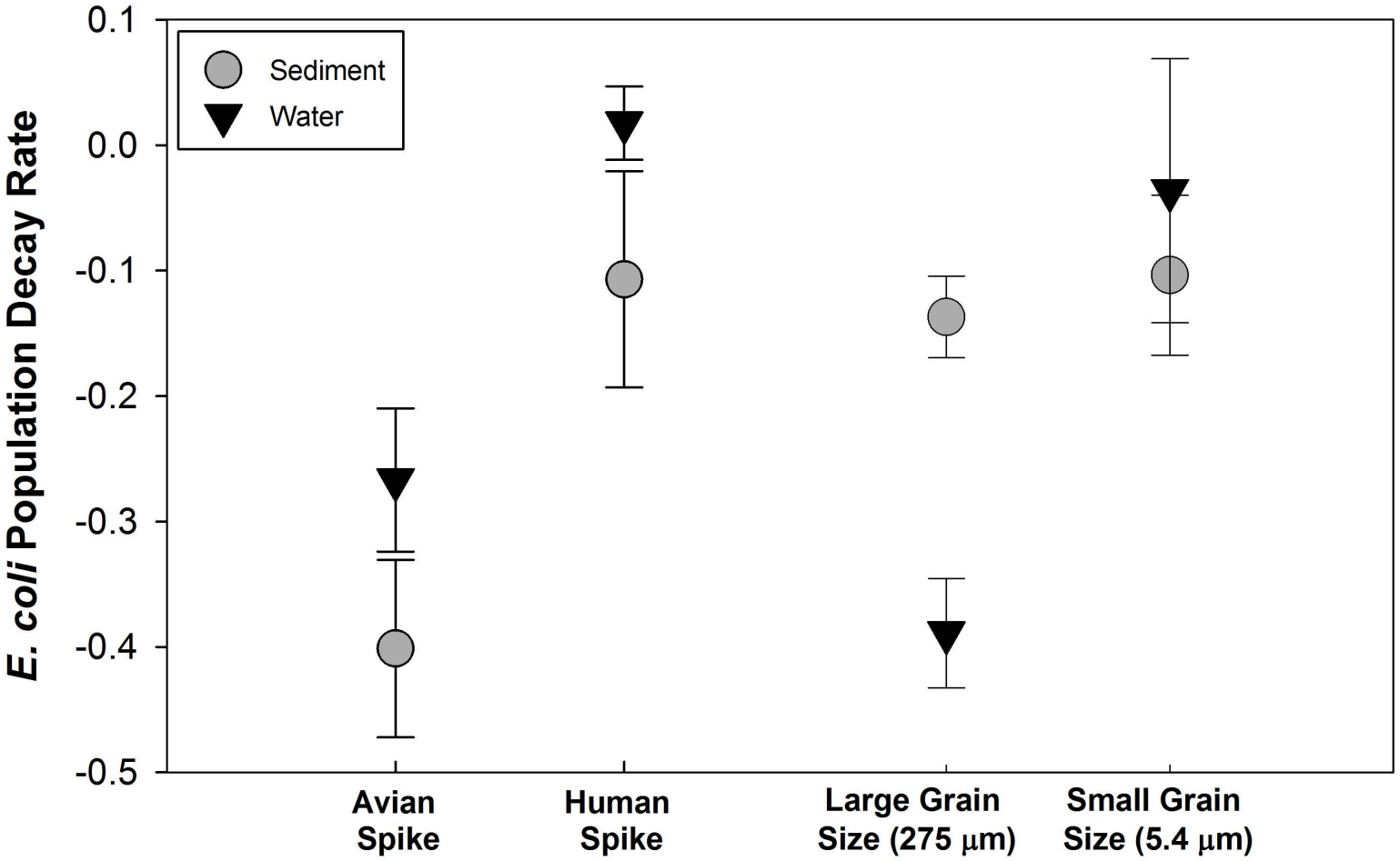

Figure 15 – Host Source Experiment *E. coli* population decay with standard error. Host source significantly affected *E. coli* population decay rates in sediment (p=0.01) and overlying waters (p=0.001). Absolute value indicates magnitude of change. Negative values indicate loss in viable bacteria.

Chapter 4 : Conclusions and future research

4.1 Research objectives

The goal of this research was to further the understanding of bacterial accumulation, persistence, and transport within a watershed. Using a combination of field and laboratory techniques we investigated the prevalence of the FIB *E. coli* in drainage basin sediments and their ability to contribute to microbial contamination of overlying waters. Results of this research indicated the extended persistence of *E. coli* across a variety of physical sediment characteristics and weather conditions. Laboratory studies indicated that host source of FIB and the physical characteristics of sediments into which they are deposited played a role in dictating their survival. Findings of both field and laboratory studies suggested a remobilization of bacteria-laden sediment during wet conditions, which likely impacted water quality. The results of these studies thus implicate drainage basin sediments as a source and sink for FIB within a watershed. These findings are of value to watershed managers as they provide evidence that FIB, such as *E. coli,* can survive for extended periods outside the host organism and thus may not be a reliable indicator of recent microbial contamination.

4.2 Watershed investigations

Field research into FIB prevalence in the Withers Swash watershed involved sampling sediments and stormwater under dry and wet weather conditions. Results of field measurements showed substantial concentrations of the FIB *E. coli* present in drainage basin sediments. *E. coli* concentrations observed during dry weather samples and rain event profiles indicated the accumulation of a persistent population in the sediment environment during dry conditions. It was hypothesized that these upper layer sediments with high FIB concentrations were remobilized during wet weather flow. BMP efficiency testing and rain event profile data provided evidence for the resuspension of these sediment-adsorbed bacteria and their contribution to overlying waters as flows increase with rainfall. Thus it is determined that sediments play an important role in the survival and transport of *E. coli* within the Withers Swash watershed. Normalizing sediment and

stormwater FIB measures and comparing them in terms of surface area (MPN m^{-2}) confirms the importance of sediments as a reservoir of significant bacterial accumulation during dry conditions. Under dry weather conditions prior to and closely following rainfall the sediment environment comprises a significant portion of the overall *E. coli* population found in watershed sediments and stormwater. These findings support previous work in the field of microbial contamination of watersheds and provide additional confirmation for the accumulation of FIB in watershed sediments via deposition (Byappanahalli et al., 2004; Jeng et al., 2005; Badgley et al, 2011), and their remobilization via erosion and resuspension (Jamieson et al., 2005; Fries et al., 2006; Chandran et al., 2011). A previously unreported finding of this study is the potential for a steady state population of FIB in watershed sediments. Post rain sediment *E. coli* data show an interesting rebound of sediment concentrations to levels similar to those recorded prior to rainfall. This trend was observed in about 66% of profiles conducted (n=28) and in at least one profile for all sites in the study area. These data suggest the trend of accumulation, erosion, and population recovery may be widespread within the drainage basin, being periodically masked by antecedent weather condition or site specific characteristics (e.g. the presence of a BMP). For sites with limited flow this implies the majority of FIB recruitment to sediments occurs immediately after rainfall, on the falling limb on the hydrograph. For sites with consistent flow during dry conditions an initial input of upstream bacteria as storm level flows recede may be followed by regular upstream contribution of freely floating or sediment-associated FIB.

The implications of this field work for microbial research within watersheds include the need to further investigate the ubiquity of sustained FIB populations in environments beyond the water column. For this study a novel sampling strategy is employed which is useful in preliminary identification of *E. coli* population rebound in sediments. Profiling rain events allows for an approximation of FIB activity throughout the course of a storm by sampling sediments and stormwater before, during, and after rainfall. This method is beneficial as it provides insight into microbial activity and a basic indication of sites which experience significant sediment contributions. While it does not attempt to quantify sediment inputs it does provide a simple means of identifying the prevalence and mobilization of FIB within a watershed.

An additional method for examining FIB adsorption and transport using FIB tests, which was successfully employed for this study, involves BMP efficiency testing. BMP retention ponds reduce runoff velocity and allow deposition of sediment particles. As a result, if a significant portion of *E. coli* are adsorbed to sediment particles, sites downstream of BMP ponds should show reduced FIB concentrations due to the deposition of sediment and associated *E. coli.* Removal of *E. coli* via sediment deposition was hypothesized based on the small mass of freely floating bacteria preventing their deposition without the added mass of a sediment particle. This method proved effective, with sites at BMP outfalls (n=4) showing significantly reduced *E. coli* concentrations in sediments and the water column when compared with those having no BMP (n=10). Thus sediments were thought to transport a significant portion of *E. coli* in the Withers Swash watershed. We believe this method is a relatively simple and effective way to demonstrate the importance of additional means of bacterial persistence, transport, and contribution to stormwater.

4.3 Microcosm studies

Microcosm studies used to examine *E. coli* colonization of sediments and overlying waters in the absence of flow showed an extended bacterial persistence in sediments relative to the water column in both experiments. These findings were consistent with previous studies emphasizing the ability of various FIB to survive for longer periods in the sediments of a variety of aquatic environments. What is historically less understood is their exchange with overlying waters in the absence of flow. Results from the Host Source Experiment show *E. coli* originating in sediments entering, and persisting in initially sterile waters. While *E. coli* are motile, results of analyses comparing bacterial concentration and population decay rate for each matrix in terms of grain size suggest their prevalence and persistence in the water column may be indirectly controlled by grain size, not die off. Sediment grain size plays a role in controlling erodibility and our findings suggest erodibility may then influence *E. coli* concentration and persistence in overlying waters. Results show small grain size sediment microcosms associated with significantly greater *E. coli* concentrations in the water column of the Water Column Colonization

Experiment and reduced decay rates for *E. coli* populations in both experiments. These findings, along with the shape of water column decay curves suggest inadvertent erosion of a small grain size sediments and adsorbed bacteria during sediment sampling contributed to water column concentrations of subsequent water samples. This suggests sediment characteristics such as grain size and organic content may not only influence *E. coli* persistence in sediments but help dictate the extent of contribution to overlying waters during times of minimal flow.

Host source of FIB was also examined as a potential factor influencing bacterial persistence in the environment. Results of the Host Source Experiment show a significant host effect on sediment *E. coli* concentrations and population decay rates in both sediments and the water column. These experiments use sterile sediments and stormwater spiked with waste from human and avian sources. Results show human source FIB colonized the sediment environment more successfully and decayed at a slower rate in sediments and the water column. These findings suggest the importance of host source upon initial survival outside the host organism. The variety of *E. coli* strains and physical make up of waste material as a vector for bacterial delivery have been suggested as reasons for the variability in bacterial survival outside the host organism. Comparing these findings with those from the Water Column Colonization Experiment suggests initial survival in sediments may be largely governed by host source while extended persistence could rely more heavily on environmental controls such as grain size and access to nutrients via organic matter. The Water Column Colonization Experiment showed grain size as a primary control of existing *E. coli* populations found in environmental sediments. It is believed that these populations have experienced the influence of host source and now see those *E. coli* that survive initial deposition persisting in higher concentrations in smaller grain size, more organic rich sediments. The Host Source Experiment more closely mimicked the process of initial FIB deposition. This experiment used sterile sediments and found that initial colonization from the water column was host dependent regardless of grain size, suggesting the importance of host source for short term *E. coli* survival.

These findings stress the complexity of microbial interactions within aquatic environments. They also suggest sediment environments may contribute to water column FIB

concentrations during dry conditions associated with minimal flow. We use sediment fall velocity to estimate the fraction of particles most likely to remain in suspension for extended periods and potentially influence water column *E. coli* concentrations. While the bacterial enumeration method was unable to quantify the portion of *E. coli* contributed by sediments, this technique provides a means of approximating the size class of sediments for a location with the ability to remain in suspension and affect water column FIB concentrations.

4.4 Implications for watershed management

The key findings of these studies further the understanding of microbial activity in a watershed and question the efficacy of FIB as a measure of microbial water quality. Field measurements and laboratory studies provide evidence for the persistence of FIB, such as *E. coli,* in the environment, particularly in drainage basin sediments. Their utility as an indicator of pathogenic species of concern relies upon their ability to detect recently deposited waste material. Results from this research suggest this is likely confounded by the long-term survival of FIB in watershed sediments. As typical bacteria tests cannot distinguish between recently deposited and long-survived FIB their results are difficult to interpret if not altogether misleading. Because FIB are intended to indicate pathogens via the recent addition of waste material, uncertainty in detecting either recently deposited or persisting FIB could results in the false detection of pathogenic organism in a waterbody which may no longer be present. This conclusion is supported by previous studies (Noble and Fuhrman 2001; Lemarchand and Lebaron 2003; Harwood et al., 2005) and provides further confirmation for FIB persistence. This research also provides new evidence of stable *E. coli* populations in sediments which may be regularly contributing to water column concentrations. While colonization of and persistence in the water column is believed to be government by erosion of small grain size sediments in this study, the mechanism may be less important to watershed managers than the finding that previously sterile water can be contaminated by FIB found in the sediment environment given minimal agitation. These results suggest that FIB in found in water column samples may have originated from recent upstream waste material, making them a reliable indicator of health concerns, or from

suspension of upper layer sediments and long survived bacteria, incorrectly indicating the presence of pathogens. Thus it is important to understand these factors controlling FIB colonization and persistence in order to accurately assess water quality.

Additional implications are related to the generation of total maximum daily loads (TMDL) designed to quantify acceptable levels for contaminants of concern which allow a waterbody to still meet quality standards. A critical part of this process is determining typical environmental concentrations for the contaminant of interest and allocating portions of the total load by source. FIB's are subject to this process and as a result an acceptable daily load must be determined of each waterbody requiring a TMDL. This may be difficult to accurately determine as water column concentrations alternate between relatively low bacterial concentrations during dry conditions and storm events which results in high FIB values. Results of this research suggest this process may also require consideration of the sediment environment as a significant additional source of bacterial input.

4.5 Future areas of research

The findings of this research contribute to the field of study as well as spark the need for additional areas of investigation. Microcosm experiments estimate the fraction of sediment responsible for bacterial contribution based on sediment fall velocity and water column height. Quantifying this value could prove beneficial in understanding the factors controlling sediment water column exchange of bacteria. One suggested means of doing this may involve the use of a method outlined by Gust and Muller (1997) to examine specific sheer stresses required for erosion of various particle sizes. Making these measurements on environmental sediments and examining bacterial concentration at each erodibility interval could prove useful in determining partitioning of FIB in poorly sorted sediments. These findings could be applied to field research using estimates of particles settling times in an attempt to better quantify contributions of sediment bacterial reservoirs.

Further research may also be focused on improving stormwater drainage systems in light of current studies suggesting the influence of drainage basin sediments on water quality within

the watershed. Watershed management research might focus on techniques that retain sediments in an effort to minimize downstream contributions of suspended bacteria. This could include the use of vegetated drainage ditches to reduce stream velocity and thus erosion of bacteria laden sediments. Two-stage ditching may also be a method for achieving a more natural state which includes a lower section with vegetation to absorb water and reduce flow velocity as well as an upper, wider section to maintain slower stormwater velocity as water levels rise.

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